SCOGS-118

EVALUATION OF THE HEALTH ASPECTS OF VITAMIN A, VITAMIN A ACETATE, AND VITAMIN A PALMITATE AS FOOD INGREDIENTS

1980

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004
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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB
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I. INTRODUCTION

This report concerns the health aspects of using vitamin A, vitamin A acetate, and vitamin A palmitate as food ingredients. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1)*, which summarizes the world's scientific literature from 1920 through 1973. In addition, the Select Committee was provided with a review prepared by LSRO (2)* of more recent literature on the health aspects of these three forms of vitamin A as food ingredients. To assure completeness and currency as of the date of this report, this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, announcement was made in the Federal Register on August 28, 1979 (44 FR 50412) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information and views on the health aspects of using vitamin A, vitamin A acetate, and vitamin A palmitate as food ingredients. Three requests were received. The Select Committee held a hearing on November 19, 1979. Those who requested opportunity to present data, information, and views are identified on page 63. The material presented at the hearing and received subsequently, have been considered by the Select Committee in reaching its final conclusions.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarket clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (3) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing, or for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects

*The documents (PB-241 949 and PB-275 754) are available from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161.
in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (3) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that these conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on vitamin A, vitamin A acetate, and vitamin A palmitate, and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.
II. BACKGROUND INFORMATION

In 1913, McCollum and Davis (4) and Osborne and Mendel (5) independently reported that an unidentified fat-soluble factor was essential for normal growth of the rat. This substance, later termed vitamin A (6), is now known to be required by all animals thus far investigated, including man (7). Dietary lack of substances possessing vitamin A activity gives rise to a deficiency state (hypovitaminosis A) in which impaired dark adaptation is the earliest sign of deficiency, followed by conjunctival and corneal involvement (xerophthalmia), and other less well defined changes. In early stages of deficiency, xerophthalmia can be reversed without residual effects by administration of vitamin A. Prolonged deficiency, even after therapy, can result in permanent impairment of vision ranging from diminution of visual acuity to total loss of sight and may carry a mortality of 25 percent or higher (8,9). Other effects attributed to vitamin A deficiency include loss of cell membrane integrity, alterations in major metabolic pathways, hormone function, and reproduction, as well as defects in the growth, development and differentiation of bone and epithelial tissues (10). Many studies in recent years (11,12) point to the probable involvement of vitamin A in a number of metabolic processes of which our understanding of some, even now, is incomplete.

Vitamin A (retinol) is the alcohol 9,13-dimethyl-7-(1,1,5-trimethyl-6-cyclohexen-5-y1)-7,9,11,13-nonatetraen-15-ol. An isomer, vitamin A₂ (retinol₂) which occurs in fresh water fishes, is 3,4 dehydroretinol. The correct chemical structure of vitamin A was proposed by Karrer et al. in 1931 (13) and it was first synthesized by Isler et al. in 1947 (14). The structure and numbering system (Figure I) was recommended by the Commission on the Nomenclature of Biological Chemistry of the International Union of Pure and Applied Chemistry (IUPAC) and has since been generally adopted (16). The IUPAC also recommended that pure vitamin A alcohol be called retinol. This recommendation has been adopted by the Committees on Nomenclature of the International Union of Nutritional Sciences and of the American Institute of Nutrition. These Committees further recommended that the term vitamin A should be used as the generic descriptor for all beta-ionone derivatives (other than provitamin A carotenoids) exhibiting qualitatively the biological activity of retinol. Thus, it should be reserved for such phrases as "vitamin A activity," "vitamin A deficiency," and the like (17).

The relationship of retinol to several other compounds with vitamin A or provitamin A activity is illustrated in Figure I. Retinyl acetate and palmitate are esters commonly used to supplement foods with vitamin A activity, β-carotene is the primary plant source of provitamin A activity, and retinal and
Structure of Retinol, Retinol₂, Retinyl Acetate, Retinyl Palmitate, Retinal, Retinoic Acid, and β-Carotene

Retinol, \( R = \text{CH}_2\text{OH} \)

Retinol₂, \( R = \text{CH}_2\text{OH} \), additional double bond at 3,4 in the ring

Retinyl acetate, \( R = \text{CH}_2\text{O} - \text{C-CH} \)

Retinyl palmitate, \( R = \text{CH}_2\text{O} - (\text{CH}_2)_{14} - \text{CH}_3 \)

Retinal, \( R = \text{C}^\text{O}_\text{H} \)

Retinoic acid, \( R = \text{C}^\text{O}_\text{OH} \)

(all-trans isomers)\(^1\)

β-carotene
(all-trans isomer)

---

\(^1\) The 4 double bonds in the side chain of retinol and its esters, and of retinal and retinoic acid can give rise to 16 possible cis-trans isomers. None has higher vitamin A activity than the all-trans isomers (15).
retinoic acid are two oxidation products of retinol. There is no
evidence that retinol2, retinol, or retinoic acid are added to
foods to supply vitamin A activity. It has been demonstrated that
rats convert β-carotene to two molecules of retinal by oxidative
cleavage at the 15,15' double bond by β-carotene 15-15' diol-
genase, an enzyme present in the intestine and liver (18,19), or
by oxidative degradation of the carbon chain to yield only one
molecule of vitamin A (20). The reduction of retinal to retinol
is catalyzed by another intestinal mucosal enzyme, retinaldehyde
reductase (21). Retinal and retinoic acid also exhibit vitamin A
activity (20,22). The all-trans isomers of the preformed vitamins
and of the provitamin A carotenoids are the predominant ones found
naturally (15). However, four synthetic mono- or di-cis isomers
of retinal have been shown to possess from 19 to 100 percent of
the vitamin A activity of all-trans retinal (23). Even axeroph-
thenol, the hydrocarbon analog of retinol, has been shown to sup-
port growth of vitamin A-deficient hamsters, but was less effec-
tive and less toxic than retinyl acetate (24).

In addition to β-carotene, several other all-trans carot-
enoids are known to possess varying degrees of provitamin A activ-
ity (25,26). Preformed vitamin A (retinol and its esters) is
found almost exclusively in animals where it is concentrated
chiefly in the liver with lesser but significant pools in the kid-
ney, milk, and blood plasma. Because dietary vitamin A activity
is supplied both as preformed vitamin A (animal products) and
provitamin A (plant products), its biopotency in foodstuffs is
usually expressed in International Units (IU) representing total
vitamin A and provitamin A activity. The IU equals 0.30 μg
retinol, 0.34 μg retinyl acetate, 0.55 μg retinyl palmitate,
0.60 μg of all-trans β-carotene, and 1.2 μg of other provitamin A
carotenoids (27,28).

Total vitamin A activities of selected foods expressed as
IU are given in Table I. It is to be noted that the Food and
Nutrition Board (28) believes that food analyses in the future
should list separately retinol, carotene, and other provitamin A
carotenoids so that "retinol equivalents" can be calculated there-
from. Since the retinol equivalent corrects for the variable
utilization of dietary carotenoids compared with retinol, it is
regarded as a preferable measure of vitamin A activity. By defini-
tion, one retinol equivalent equals 1 μg retinol, 6 μg carotene,
and 12 μg other provitamin A carotenoids. In terms of IU, one
retinol equivalent equals 3.33 IU of retinol, and 10 IU of
β-carotene.

The Code of Federal Regulations (3) classifies vitamin A
[21 CFR 182.5930], vitamin A acetate [21 CFR 182.5933], and vi-
tamin A palmitate [21 CFR 182.5936] as generally recognized as safe
(<<GRAS>>) for addition to foods as nutrients and/or dietary supple-
ments. Only these three sources of vitamin A activity are eval-
uated in this report. The provitamin A, carotene [21 CFR 182.5245],
TABLE I

Vitamin A Activity of Selected Foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Vitamin A Activity (IU per 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal products</strong></td>
<td></td>
</tr>
<tr>
<td>Beef, total edible, raw</td>
<td>30-50</td>
</tr>
<tr>
<td>Chicken meat, raw</td>
<td>60-150</td>
</tr>
<tr>
<td>Salmon, canned</td>
<td>60-230</td>
</tr>
<tr>
<td>Milk, cow, whole</td>
<td>140-150</td>
</tr>
<tr>
<td>Milk, human</td>
<td>240</td>
</tr>
<tr>
<td>Oysters, raw</td>
<td>310</td>
</tr>
<tr>
<td>Cream, fluid</td>
<td>480-1,540</td>
</tr>
<tr>
<td>Eggs, whole</td>
<td>1,180</td>
</tr>
<tr>
<td>Cheese, cheddar</td>
<td>1,310</td>
</tr>
<tr>
<td>Butter</td>
<td>3,300</td>
</tr>
<tr>
<td>Liver, calf, raw</td>
<td>22,500</td>
</tr>
<tr>
<td><strong>Vegetable products</strong></td>
<td></td>
</tr>
<tr>
<td>Apples, unpared</td>
<td>90</td>
</tr>
<tr>
<td>Bananas, common</td>
<td>190</td>
</tr>
<tr>
<td>Oranges, peeled</td>
<td>200</td>
</tr>
<tr>
<td>Lettuce</td>
<td>330-1,900</td>
</tr>
<tr>
<td>Beans, green, boiled</td>
<td>540</td>
</tr>
<tr>
<td>Peas, green, boiled</td>
<td>540</td>
</tr>
<tr>
<td>Broccoli, spears, boiled</td>
<td>2,500</td>
</tr>
<tr>
<td>Apricots, raw</td>
<td>2,700</td>
</tr>
<tr>
<td>Cantaloupes, orange</td>
<td>3,400</td>
</tr>
<tr>
<td>Spinach, boiled</td>
<td>8,100</td>
</tr>
<tr>
<td>Carrots, boiled</td>
<td>10,500</td>
</tr>
</tbody>
</table>

*Excerpted from reference 29.
which is also included in this section of the Code, will be evaluated in a separate report of the Select Committee (30). No other preformed or provitamin A source is considered GRAS and, so far as is known by the Select Committee, is not currently used for supplementation of foods.

In commercial practice most of the vitamin A supplementation of foods is accomplished by adding, together with antioxidants, synthetic retinyl acetate or palmitate, or mixtures of the two, in the form of oil solutions, water emulsions, or dried emulsions (beadlets) in a water dispersable matrix. Synthetic retinol is also used but to a lesser extent. All are synthesized as the all-trans isomers. Since cis-trans isomerization of the side chain double bonds of vitamin A (Figure I) occurs readily on heating or exposure to light with concomitant reduction in vitamin A activity, vitamin A palmitate is also offered commercially as the pre-isomerized equilibrium cis-trans mixture. Both the acetate and palmitate esters are more heat stable than retinol, making them preferable for many uses. The palmitate is more stable than the acetate in the presence of moisture. Foods receiving severe heat treatment during processing, e.g. breakfast cereals, are usually fortified subsequent to heating by spraying an oil/water emulsion onto the surface of the processed product. However, vitamin A is quite stable during the baking of bread, 90 to 100 percent being retained after baking, and 85 to 95 percent after storage of the bread for 5 days at room temperature (22).

Retinol occurs as yellow prisms melting at 62 to 64°C, has a molecular weight of 286, is water insoluble but soluble in alcohol, ether, acetone, benzene, and in fats and oils. Retinyl acetate occurs as pale yellow prisms, melts at 57 to 58°C, has a molecular weight of 328, and has solubilities similar to retinol. Retinyl palmitate, occurring either in amorphous form or as yellow crystals, melts at 28 to 29°C, has a molecular weight of 524, and also has solubilities similar to retinol (1,31).

According to the Food Chemicals Codex (32), food grade vitamin A consists of retinol or retinyl esters formed from edible fatty acids (primarily acetic and palmitic acids). The vitamin may be diluted with edible oils or may be incorporated in solid edible carriers, extenders, or excipients; it may contain suitable preservatives, dispersants, and antioxidants provided the product is not used in foods in which such substances are prohibited. The product must assay not less than 95 percent of the vitamin A activity declared on the label, be stored in tight containers, preferably under an atmosphere of inert gas, and protected from light. The product should be labeled to indicate the form of the vitamin, its potency in both milligrams retinol and United States Pharmacopeia (USP) units, and the presence of any preservative, dispersant, antioxidant, or other added substance.
III. CONSUMER EXPOSURE DATA

Based on surveys made in 1971 to 1974, the Health and Nutrition Examination Survey (HANES) (33) on the dietary intake of various nutrients in a representative sample of the U.S. population (all incomes and both sexes), showed that the mean daily intake of vitamin A from all food sources was 4,774 IU for ages 1 to 74 and 4,500 IU for ages 20 to 24 yr. However, about 47 percent of the latter group was found to consume less than 3,500 IU daily (34). This survey assumed the vitamin A activity in the daily diet was 70 percent as provitamin A (carotene) and 30 percent as preformed vitamin A (retinol). Intake of vitamin A from prescription or over-the-counter vitamin preparations was excluded. It is to be noted that the 1974 recommended daily dietary allowance (RDA) of vitamin A (28) was 5,000 IU for adults; 1,400 to 2,000 IU for infants; 2,000 to 3,300 IU for children up to age 11; 5,000 IU for pregnant and 6,000 IU for lactating women. In the 1980 revision of the RDA for vitamin A (35), the National Research Council (NRC) has reduced recommended daily dietary allowances to 2,600 to 3,300 IU for adults, 1,300 to 1,400 IU for infants, and 1,300 to 2,300 IU for children. For comparison, the Joint Food and Agriculture/World Health Organization (FAO/WHO) in 1967 recommended the following daily intakes: adults, 750 μg retinol (2,500 IU); infants, 300 μg retinol (1,000 IU); children up to 10,400 μg retinol (1,300 IU); pregnant women, 750 μg retinol (2,500 IU); and lactating women, 1,200 μg retinol (4,000 IU) (36).

In 1972 a NRC subcommittee (37) estimated the possible daily intake of preformed vitamin A added to food. Their estimates were based on data obtained in a survey of the food industry from which they calculated the weighted mean level of addition of retinol, retinyl acetate, and retinyl palmitate to foods by food category (Table II). Using portion size data from the U.S. Department of Agriculture, frequency of eating particular foods from the Market Research Corporation of America, and assuming that all foods in a category contained vitamin A at the levels indicated in Table II, the NRC subcommittee calculated the daily intakes of these vitamin forms for several age groups. However, as pointed out by the NRC subcommittee and as explained in Section XI of the NRC subcommittee's report (37), this procedure is likely to lead to estimates of intakes that are overstated, often by considerable margins. That this is obviously true in this instance is indicated by the following daily intakes for the 2 to 65+ yr age group, derived by that procedure: retinol, 3.25 mg (10,800 IU); retinyl acetate, 23.14 mg (70,000 IU); retinyl palmitate, 11.06 mg (20,000 IU).
TABLE II

Average Level of Addition of Retinol, Retinyl Acetate, and Retinyl Palmitate to Foods by Food Category (37)*

<table>
<thead>
<tr>
<th>Food category</th>
<th>Retinol</th>
<th>Retinyl acetate</th>
<th>Retinyl palmitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weighted mean, mg per kg (ppm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baked goods, baking mixes</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>1.0</td>
<td>48.0</td>
<td></td>
</tr>
<tr>
<td>Grain products such as pastas or rice dishes</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Fats and oils</td>
<td>6.3</td>
<td>9.5</td>
<td>15.7</td>
</tr>
<tr>
<td>Milk products</td>
<td>0.6</td>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td></td>
<td></td>
<td>25.0</td>
</tr>
<tr>
<td>Meat products</td>
<td></td>
<td></td>
<td>6.1</td>
</tr>
<tr>
<td>Poultry products</td>
<td></td>
<td></td>
<td>6.1</td>
</tr>
<tr>
<td>Processed vegetables, juices</td>
<td>270.0</td>
<td></td>
<td>1.9</td>
</tr>
<tr>
<td>Soft candy</td>
<td></td>
<td>200.0</td>
<td></td>
</tr>
<tr>
<td>Sweet sauces, toppings, syrups</td>
<td></td>
<td>26.1</td>
<td></td>
</tr>
<tr>
<td>Snack foods</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Beverages, non-alcoholic</td>
<td>30.0</td>
<td></td>
<td>57.6</td>
</tr>
<tr>
<td>Nuts, nut products</td>
<td></td>
<td></td>
<td>180.0</td>
</tr>
<tr>
<td>Dairy product analogs</td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Baby food formulas</td>
<td></td>
<td></td>
<td>2.5</td>
</tr>
</tbody>
</table>

*The NRC subcommittee reported levels of addition in weighted mean percent. The Select Committee has converted these to ppm (mg per kg of food) as being more meaningful in this context. One mg of retinol equals 3,333 IU; of retinyl acetate, 2,940 IU; and of retinyl palmitate, 1,820 IU.
The NRC subcommittee (38), in a recent survey of appropriate segments of the food industry, has found that those responsible for most of the vitamin A supplementation of foods in 1975, used a total of 9,150 kg of retinol, 83,200 kg of retinyl palmitate, and negligible amounts of retinyl acetate. This amounted to nearly 2,000 IU per capita daily of added vitamin A. However, the Select Committee believes this figure to be substantially higher than actual per capita intake. The HANES survey (33) cited above indicated a daily intake of 5,000 IU vitamin A from all sources (natural and added preformed vitamin A; natural and added carotene). Based on the 1966 estimate of Greaves and Tan (39), about two-thirds of the daily intake is provided by carotene, leaving a balance of about one-third to be provided by that added and that naturally present. This one-third would be equivalent to about 1,700 IU. Obviously, the amount of added preformed vitamin A in the daily diet must be substantially less than the estimated 1,700 IU. Based on some rough estimates of the amounts of vitamin A that would be required in the most commonly fortified foods, the Select Committee believes that the per capita intake of vitamin A, due to that added to foods, is about 800 IU daily.
IV. BIOLOGICAL STUDIES

Absorption, metabolism, excretion

Oral doses of retinol are readily absorbed. In vitamin A, depleted rats each fed 3 mg retinol (10,000 IU), serum levels of retinyl esters reached a maximum in about 5 h. After 24 h virtually all vitamin A activity was found in the liver. Vitamin A activity in blood and liver was present primarily as the palmitate ester although small amounts were also found of the stearate, myristate, and laurate esters (40). The dosage form significantly affects the rate of absorption of vitamin A preparations. Nishigaki et al. (41) found 10 mg of retinyl palmitate (18,000 IU) fed to rats in aqueous emulsion reached maximum concentrations in the lymph in 1 to 2 h, but required 4 h to peak when the same dose was given as an oily emulsion. In a patient with a cannulated thoracic duct, 78 percent of the radioactivity of fed $^{14}\text{C}$-labeled retinol (at carbon 15) was found in the lymph between 2 and 8 h, with a peak between 3 and 5 h (42). In normal men and women, vitamin A levels in blood serum were maximal within 4 to 6 h after ingestion of a single dose of 134 mg whether administered as the alcohol, acetate or natural esters (43). Schneebberger et al. (44) found that serum levels of vitamin A peaked at about 400 $\mu$g per 100 ml after 3 to 4 h in eight men fed 150,000 IU (2,500 IU per kg) of retinyl palmitate, returning to normal levels within 24 h. Pereira and Begum (45) fed six Indian children 100,000 IU retinyl palmitate together with 11,12 $^3\text{H}$-labeled retinyl acetate as part of a meal and found serum retinol increased sharply 4 h after dosing and declined during the following week. Monitoring of fecal and urinary excretion indicated a retention of 23 to 54 percent of the dose after 8 days.

Absorption of ingested retinol occurs mainly in the mucosal cells of the intestine and is enhanced by bile (12). Small amounts may be absorbed from the stomach since about 0.1 percent of an intubated dose of $^{14}\text{C}$-labeled retinol or retinyl acetate was found after 2 h in the liver of rats with subpylorically ligated stomachs (46). In most species, retinyl esters, such as retinyl acetate or palmitate, are first hydrolyzed in the lumen of the intestine by retinyl ester hydrolases from the pancreas (47) and within the outer brush border of the intestinal mucosal cell (48). After absorption of retinol by the mucosal cell it is reesterified (catalyzed by retinyl ester synthetase) mainly to retinyl palmitate (40,48) which enters the circulation principally via the chylomicron fraction of the lymph and is stored as the palmitate in the liver (42).

Prior to re-entering the blood, the hepatic retinyl ester is hydrolyzed and the retinol is bound to a specific transport protein, retinol-binding protein (RBP) (49), which occurs in free
form in human serum (50) and which, in the plasma, is further complexed, in a molar ratio of 1:1, with a prealbumin (51). This prealbumin appears to be similar, if not identical, to the prealbumin which binds thyroxine (11,50).

The RBP's of man, rat, and dog have similar molecular weights (about 20,000), but a high degree of immunological specificity exists within a given mammalian order (52). By contrast, Rask (53) found porcine RBP, molecular weight about 21,000, to show no immunological cross-reactivity with the corresponding RBP's of the rat, monkey, or man. Vahlquist (54), using iodine-labeled and endogenously 35S-labeled proteins as tracers in the monkey, estimated biological half-times of 1.9 h, 6.6 h, and 22.5 h for RBP in free form, RBP-prealbumin complex, and prealbumin itself, respectively.

The RBP-retinol-prealbumin complex is believed to prevent the circulating RBP (and retinol) from glomerular filtration of retinol and excretion by the kidney (12). It is postulated that retinol transport proceeds via dissociation of the RBP-retinol complex from the prealbumin, followed by release of retinol from RBP, deposition of retinol in a target cell, and excretion of free RBP in the urine (55). Delivery of retinol to extra-hepatic tissues appears to involve specific cell surface receptors for RBP (56). In some species, notably fish and eels, retinol appears to be transported by uncomplexed RBP-retinol or as retinyl palmitate attached to a high molecular weight lipoprotein (57). Other recent studies have provided evidence for the existence of a cellular RBP distinct from plasma RBP, and of another cellular binding protein specific for retinoic acid with no affinity for retinol (58-60).

Linder et al. (61) have shown, contrary to earlier conclusions, that the principal storage site of retinol in rats is in the hepatocytes of the liver (more than 90 percent) while less than 10 percent is in the Kupffer cells. They suggest, however, that the possibility cannot be excluded that the Kupffer cells are the initial recipients of vitamin A, subsequently transferring it to the hepatocytes. Sundaresan (11) has postulated further that hepatocytes may store retinyl esters while the Kupffer cells store the free alcohol. Ames et al. (62) found in rats that over 50 percent of an ingested daily dose of up to 120,000 IU per kg as vitamin A acetate was stored in the liver by the 28th day, and concluded that liver apparently reaches maximal vitamin A storage at about 50,000 IU per g of fresh liver.

Some investigators (63,64) believe that stored retinyl esters from the liver are released by retinyl palmitate hydrolase found in the nuclear and mitochondrial fractions of rat liver and by retinyl acetate hydrolase which has been isolated from rat liver microsomes. However, because Yeung and Veen-Baigent (65)
were unable to demonstrate retinyl palmitate hydrolase in liver homogenates or powders, they have continued to maintain that retinol is formed from retinyl palmitate by extrahepatic hydrol-
ysis. Thus, the actual mechanism(s) for the hydrolysis of stored retinyl esters remains in doubt. Moreover, at least two other observations must be taken into account in the ultimate unraveling of the sequence of steps and sites involved in the metabolism of vitamin A. Sewell et al. (66) found in rats that even though total vitamin A activity in the liver remained constant for a period of 45 days following oral administration of retinyl acetate labeled with tritium on carbons 15 and 16, radioactivity in the liver progressively decreased. Similar results were obtained in steers (67). The estimated half-time of vitamin A in the liver was 57 days in rats and 48 days in steers. The investigators (66,67) concluded that liver vitamin A stores are in a dynamic state with a continuous turnover of vitamin A reserves, even though the total storage remains essentially constant. Retinol and some of its derivatives cycle between the liver and the gut as enterohepatic circulation (68). About 20, 30, and 60 percent, respectively, of labeled retinol, retinal, and retinoic acid injected intraperitoneally were excreted in the bile of cannulated rats within 24 h. When the excreted bile was placed in a duodenal loop of another rat, radioactivity disappeared from the gut and reappeared in the bile. Hume et al. (69), reporting similar and substantial recycling of retinol metabolites in sheep, have suggested that this may be a conservation mechanism operative during periods of retinol deprivation.

Several physiological and biochemical roles have been ascribed to vitamin A, some well established and others less so. Retinol is accepted as essential for development and maintenance of normal vision. This mechanism is well understood and has been elaborated in a number of reviews cited by Sundaresan (11) and by Mandel (12). Retinol is regarded as essential for normal growth and reproduction but the mechanisms remain obscure. Vitamin A may also be involved in biological sulfate activation, in steroid and protein biosynthesis, and in maintenance of membrane integrity (11). Recent studies point to a possible relationship between vitamin A and zinc metabolism, since zinc-deficient rats, swine, and lambs exhibit reduced plasma vitamin A and RBP, adequate concentrations of liver vitamin A, but depressed concentrations of RBP in the liver (70). These effects of vitamin A deprivation or deficiency and the mechanisms of response to the administration of vitamin A are not particularly relevant to the purposes of this report in assessing the potential for hypervitaminosis A. There is the view, however, supported by some of the older literature and dietary intake studies, that hypovitaminosis A in some seg-
ments of the population could be considerably more serious, rela-
tively, than the potential threat of hypervitaminosis A (71,72). However, Underwood (73) contends that while vitamin A deficiency is said to be one of the remaining nutrient-specific deficiency diseases of worldwide public health importance, the magnitude of
the problem and its severity on a global basis are not known. This is attributed in part to the lack of adequate indicators of the relative level of subclinical nutrure. She states further that the current means for assessment leaves wanting a definitive answer to the question of whether inadequate vitamin A nutrure is a problem of public health significance in the United States.

Whether retinol itself or a metabolic product(s) is the "active" form of the vitamin as it affects the target organs and tissues indicated above, remains in doubt. According to Sundaesran (11) and Mandel (12) it is currently considered likely that either retinoic acid or an as yet unidentified metabolite of retinoic acid is the systemically active form of the vitamin for growth and tissue maintenance, while retinal is the active form for maintenance of vision and retinol for reproduction. DeLuca and Zile (74) also summarize current evidence to indicate that retinoic acid or a metabolite is the form which carries out the growth-promoting functions of vitamin A. Enzymes capable of converting retinol to retinal and to retinoic acid exist in the liver, intestinal mucosa, and retina (12,75,76).

The normal plasma concentration of vitamin A is about 30 to 70 µg (100 to 230 IU) per 100 ml but much higher levels have been reported after vitamin A supplements are consumed (12). For example, Gerber et al. (77) measured blood levels of vitamin A in four patients with differing intakes of the vitamin. The fasting vitamin A levels of these patients per 100 ml blood and their daily intakes were, respectively: 120 µg (25,000 to 100,000 IU for 8 years); 88 µg (25,000 to 50,000 IU for 6 years); 60 µg (25,000 IU for 6 years); 225 µg (50,000 IU for 8 months). Only the first and last of these patients had complaints referable to vitamin A toxicity.

Median concentration of vitamin A in man is about 100 µg per g of liver with about 1 µg per g present in such tissues as kidney, lung, adrenal, and intraperitoneal fat. Selective localization of the vitamin occurs in the retina of the eye (12). The calculated body pools of vitamin A, as measured in four individuals by a radioisotopic method using retinyl-14C acetate, were 315, 412, 766, and 877 mg (78). Raica et al. (79) found the mean concentration of vitamin A in the liver to be 146 µg per g, SD ±151, in 372 necropsy samples taken in five different states; most other tissues contained 1 µg or less per g. Only trace amounts of β-carotene were found in the livers. Lorente and Miller (80) demonstrated that vitamin A passes the placenta and concentrates in the fetal liver in rats, but not in rabbits.

The major route of excretion of retinol and its metabolites in the rat appears to be the feces via the bile but significant amounts of some metabolites are also excreted in the urine, and carbon dioxide from oxidation of the carbons of the side chain is excreted via expired air. Roberts and DeLuca (81) injected
retinol-deficient rats intravenously with $^{14}$C-labeled retinyl acetate and retinoic acid (doses of 2.0 and 14.5 mg, respectively) and measured recovery of radioactivity in expired CO$_2$, urine, and feces 2 days after injection. With 6,7 $^{14}$C$_2$-retinyl acetate, the percentages of the dose recovered in expired CO$_2$, urine, and feces were about 2, 17, and 18, respectively; with 6,7 $^{14}$C$_2$-retinoic acid, the corresponding percentages were about 1, 38, and 64. When 15 $^{14}$C-retinyl acetate or 15 $^{14}$C-retinoic acid was injected, the corresponding percentages of radioactivity recovered were about 10, 10, and 14 for the acetate, and about 35, 20, and 44 for the acid. Since total recovery of administered dose after 2 days was substantially greater for the acid (about 100 percent) than for the acetate (about 30 percent), the authors concluded that metabolism of the acetate proceeds at a much slower rate. From these and other experiments, DeLuca and associates (81,82) have postulated at least three pathways for the ultimate excretion of retinyl acetate or retinoic acid metabolites:

I. Products containing intact isoprenoid side chain representing 60 to 80 percent of the ingested dose; excreted predominantly in the feces and the remainder in the urine, presumably as retinoyl β-glucuronic acid.

II. Products decarboxylated at carbon 15 representing about 10 to 20 percent of the ingested dose; carbon 15 will be expired as CO$_2$ and the remainder excreted in the feces.

III. Products in which some or all of the isoprenoid side chain has been oxidized to CO$_2$; the CO$_2$ will be expired and derivatives of the remainder of the molecule excreted in the urine (some 17 to 20 percent).

Similar results were obtained by Nath and Olson (83) who found that about 65 percent of an intraperitoneal dose of 15 $^{14}$C-retinoic acid was excreted in the feces of rats with about 30 percent of the radioactivity accounted for by retinoyl β-glucuronide. Lippel and Olson (84) demonstrated that retinoyl β-glucuronide is by far the major metabolite, if not the sole one, in the bile after intraportal injection of retinoic acid and suggested that the reported presence of retinoic acid esters, the γ-lactone of retinoyl β-glucuronide, and retinoic acid itself is due to reactions catalyzed by the anion-exchange resin used in the extraction process.

The nature of the urinary metabolites was explored further by Sundaresan and Bhagavan (85) who found evidence of at least six types of metabolites in the urine after injection of
physiological doses of differentially labeled retinoic acid into retinol-deficient rats. They concluded that the major metabolite(s) was one lacking both carbons 14 and 15 of retinoic acid. Retinoyl β-glucuronide was not present in the urine in significant amounts. Using improved chromatographic procedures, DeLuca and Zile (74) have detected, but not identified, at least eight metabolites of retinoic acid in tissue extracts of liver, kidney, blood, small intestine, and skin. Roberts and Frolik (86) found retinoic acid to be converted both in organ culture and in subcellular preparations from intestinal mucosa, liver, and testis to several metabolites that are more polar than retinoic acid, among them being 4-hydroxy- and 4-keto-retinoic acid. These investigators suggest that oxidative attack at carbon 4 may be the first step in the elimination of retinoic acid from the tissues.

During a study of the requirement of man for retinol in which seven subjects were injected with 10 to 15 mg of 15\textsuperscript{14}C-retinyl acetate, pulmonary excretion of \textsuperscript{14}CO\textsubscript{2} was found to be the major excretory route (87).

Acute toxicity

Ames et al. (62) estimated the oral, single dose, LD\textsubscript{50} for crystalline vitamin A acetate dissolved in cottonseed oil in the rat to be about 10 million IU (3.4 g) per kg body weight. In 28-day subchronic tests, no deaths occurred at a feeding level of 150,000 IU (51 mg) per kg per day, and an LD\textsubscript{50} at 28 days of 200,000 to 300,000 IU (68 to 102 mg) per kg per day was simultaneously estimated. They also observed that depression of growth curves closely parallel breaking strength of excised leg bones and, hence, was a very sensitive measure of vitamin A toxicity. Rats on daily supplements as high as 100,000 IU per kg showed normal growth while there was marked growth depression at levels of 150,000 to 200,000 IU per kg. No details concerning animal age or weight, dose range, or sex and number of animals per dose were provided.

McLaren et al. (88) found that only 2 of 20 adult Sprague-Dawley rats survived more than 3 days following intraperitoneal injection of 200 to 500 mg (667,000 to 1.6 million IU) of retinol per kg body weight. Similar results (only 4 of 11 animals survived) were obtained with weanling rats infected with 425 mg (1.4 million IU) retinol per kg. Survival time was inversely related to plasma retinol and retinyl ester levels. However, all 7 adult rats survived 2 months and showed no signs of toxicity after intraperitoneal injection of 370 mg (673,000 IU) of retinyl palmitate per kg body weight.

Acute poisoning resulting from consumption of polar bear liver has been attributed to its high vitamin A content (13,000 to
18,000 IU per g). In typical cases, illness, including drowsiness, irritability, headache, and vomiting followed within a few hours after a meal of polar bear liver (89-91).

In infants given single oral doses of 350,000 IU of vitamin A, transient hydrocephalus and projectile vomiting occurred about 12 h after treatment (92). Woodward et al. (93) also noted that an infant given 70,000 IU of vitamin A daily (about 20,000 IU per kg body weight) from age 4 days exhibited bulging of the fontanel at 2 months of age and suffered as well from hyperirritability, hyperesthesia, alopecia, and increased intracranial pressure. Caffey and Silverman (94) have indicated that early clinical features of vitamin A toxicity are not distinctive and it is only after 6 or more months following onset of excessive intake, when the blood level of vitamin A is definitely increased and there is tenderness, pain, and swelling of the extremities, that the clinical picture becomes diagnostic.

Hillman (95) experimentally produced and studied hyperavitaminosis A in a 40-year-old, 75 kg male. In the first experimental period, 1 million IU of water-miscible vitamin A (about 13,000 IU per kg body weight) were ingested daily for 13 days. Plasma vitamin A level peaked at about 750 μg per 100 ml on day 14 and receded to less than 100 μg per 100 ml (pretreatment level) by day 20. Principal clinical features included severe headache, chiefly frontal and retro-orbital, pruritic dermatitis, generalized desquamation, alopecia, increased fragility of the finger nails, splitting and chapping of the lips, gastrointestinal disturbance, anorexia, nausea, alternating constipation and diarrhea, visual disturbance, transitory dizziness, weakness, fatigue, and pain and tenderness in the leg bones. Similar effects on plasma level were observed in a second experimental period of 25 days at the same daily dosage. Some of the signs and symptoms experienced during the first experimental period did not appear in the second but some were more severe in the latter.

**Chronic toxicity – laboratory animals**

The adverse effects, principally characteristic bone lesions, of greatly excessive doses of vitamin A concentrates in laboratory animals were noted nearly a half century ago (96). These and other early observations were inconclusive in implicating vitamin A as the causal agent because of the presence of variable amounts of unknown impurities in some of the vitamin A preparations used. However, they were confirmed when pure retinol and its esters became available and many subsequent studies have helped to delineate the multiplicity of adverse consequences of hypervitaminosis A. Wolbach (97) has reviewed the earlier work with respect to the skeletal effects of vitamin A deficiency and excess.

Male rats fed 50,000 IU of vitamin A as retinyl acetate daily for 4 days mixed with their basal ration, and 25,000 IU daily thereafter (330,000 IU per kg body weight per day) for the
next 20 days, developed abnormally thin bones, fractures and exophthalmos in all animals and subcutaneous and intramuscular hemorrhages in some animals (98). Cappellin and Crepax (99) found that rats tolerated a daily intubated dose of 10,000 IU of retinyl acetate (about 130,000 IU per kg body weight) for 3 months without observed adverse effects. When the daily dose was doubled (about 260,000 IU per kg body weight) in another group of rats, all animals lost weight and died within 25 to 30 days. In these animals, spontaneous and multiple bone fractures occurred by the 15th day; alopecia and inflammation of connective tissue accompanied by hemorrhage were observed. Conne et al. (100) fed rats vitamin A acetate at a level of about 165,000 IU per kg body weight daily for 2 weeks during which they lost weight and became moribund. Necropsy showed increased vascularization of pulp and periodontium of the upper incisor accompanied by thinning of the dentin and hypercalcification of the enamel and dentin.

Randall (101) fed up to 50,000 IU vitamin A per day as palmitate per kg body weight to rats for 10 months and up to 25,000 IU per day per kg body weight to dogs for 10 months with no observed adverse effects on growth rates or hematolgy.

Lewis and Cohlan (102) intubated groups of 50 adult male rats with 25,000, 50,000 or 100,000 IU vitamin A (125,000 to 500,000 IU per kg body weight) daily, either with a water dispersable commercial preparation of vitamin A (Aquasol®) or with vitamin A natural esters, for 21 days. All animals receiving 100,000 IU of the Aquasol® died by the eighth day, while 83 percent of the animals receiving 100,000 IU of natural esters survived 21 days. Forty percent of the animals receiving 50,000 IU of the Aquasol® died within 21 days as contrasted with 6 percent mortality in the 50,000 IU natural ester group. During the 21 day experimental period, signs of vitamin A toxicity included scrubiness of the pelt, drowsiness, muscular weakness, reduced appetite and growth, alopecia, exophthalmos, limping, spontaneous fractures, and scoliosis. No mention is made of mortality in the animals receiving 25,000 IU daily, either as aqueous or oily preparations; presumably no obvious signs of hypervitaminosis A appeared at this level.

Ames et al. (62) found depression of growth rate to be a very sensitive measure of vitamin A toxicity. Rats showed normal growth curves with doses of retinyl acetate up to 100,000 IU per kg body weight per day but marked growth depression at levels of 150,000 to 200,000 IU per kg. Breaking strength of excised leg bones decreased with increasing dosages and closely paralleled depression of the growth curve. Organ weights after toxic doses were essentially normal with the exception of marked hypertrophy of the adrenals. Sobel et al. (103) found no growth depression in rats on stock ration supplemented by intubated retinyl acetate at doses up to 30,000 IU per kg per day but marked depression in growth rate at 300,000 IU per kg per day.
Nerurkar and Sahasrabudhe (104) fed retinyl palmitate (about 400,000 IU per kg body weight) daily to rats for periods of 3, 6, 9, and 12 consecutive days, and determined the calcium, phosphorus, nitrogen, and ash content of the bone in animals sacrificed following the final daily dose; blood serum levels of calcium and inorganic phosphorus, blood and liver levels of vitamin A, and urinary and fecal excretion of calcium, phosphorus, and nitrogen were also determined. There was reduced food intake, weight loss, and skeletal fractures and hemorrhages, with the degree of toxicity approximately proportional to the total quantity of vitamin A consumed. A negative balance was observed with respect to calcium, phosphorus, and nitrogen with the trend continuing long after cessation of administration of large doses of the vitamin. No changes were detected in the levels of calcium and inorganic phosphorus in the blood, nor in the relative mineral composition of the bones. However, bone thinning occurred accompanied by increased urinary and fecal excretion of calcium and phosphorus.

Matrajt-Denys et al. (105) found that Wistar rats, given 2,500 IU of retinyl acetate in peanut oil subcutaneously 3 times a week for 4 weeks (about 35,000 IU per kg body weight on injection days), showed reduced rate of weight gain. Femoral length and the area of cortical bone at midshaft was less than in the controls. Histological studies revealed that the caudal vertebrae had thinner cortices and less trabecular bone, calcified cartilage and growth cartilage. The number of vascular channels and the percentage of osteocytic cavities greater than 15 microns in diameter were increased. However, subcutaneous administration of porcine calcitonin to animals receiving vitamin A at the above level, partially or wholly prevented these changes. The investigators concluded that vitamin A inhibits bone growth and stimulates bone resorption while calcitonin prevents both effects.

Berdjis (106) fed 40 rats 100,000 to 300,000 IU retinol per kg body weight daily, sacrificing 10 rats each week after 1, 2, 3, and 4 weeks. Extensive hemorrhages and multiple spontaneous fractures occurred during the first 2 weeks. In surviving animals, degeneration of cartilage cells and replacement of cartilage by bone were accelerated and resulted in early closure of the epiphyses. Weight loss and similar pathological signs at exaggerated doses were also observed in rats fed about 600,000 IU per kg body weight per day of retinyl acetate for 10 days (107); about 1,000,000 IU per kg of the acetate ester for 5 days (108); or about 2,000,000 IU per kg per day of the palmitate ester for 16 days (109).

Regezi and Rowe (110) fed 10,000 IU retinol every 2 days (60,000 IU per kg body weight on days fed) to 10 male Sprague-Dawley rats for 16 weeks and 50,000 IU daily (455,000 IU per kg per day) to 5 male Sprague-Dawley rats for 16 days. Rats on the lower dosage grew as well as the controls up to the fourteenth
week, at which time they began to lose weight. The difference in mean weights between experimental and control animals was not statistically significant until the sixteenth week and the animals were normal in appearance and behavior. Rats on the higher dosage, on the other hand, had progressive weight loss beginning at day 6, were lethargic and inactive, and lost hair. Morphologic abnormalities occurred in the submandibular gland at both dose levels but were more pronounced at the higher level.

Leelaprute et al. (111) fed groups of 10 female rats 25,000, 50,000, and 75,000 IU of retinol daily (about 160,000 to 480,000 IU per kg). Animals at the 25,000 IU retinol dose level and the 50,000 IU retinyl palmitate level failed to gain weight but showed no gross bone changes or microscopic calcification of kidneys, lungs, heart, or liver; all animals lost weight at higher dose levels and most showed gross bone changes and organ calcification.

Seawright et al. (112) found dietary levels of about 54,000 and 270,000 IU vitamin A per kg body weight as the palmitate for 24 to 41 weeks produced primary lesions mainly in the first three joints of the cervical vertebrae of cats. There was extensive osseocartilagenous hyperplasia at the margins of the joints. These changes did not occur in control animals receiving no supplemental vitamin A or at a dietary level of 27,000 IU vitamin A per kg body weight. Clark (113) fed weanling kittens retinyl acetate (approximately 210,000 IU per kg per day) for 21 to 30 days and found food consumption to be reduced by 20 to 80 percent. Reduced endochondral bone growth occurred because of osteoporosis and damage to the epiphyseal plates.

Male, Hartley strain guinea pigs receiving in the diet up to approximately 4,000 IU vitamin A as retinol per kg per day for 40 to 50 days maintained normal growth with no evidence of toxicity, while a dose of about 130,000 IU per kg for the same period was toxic and caused depressed growth rate (114).

Weanling crossbred boar pigs fed up to about 20,000 IU of retinyl acetate per kg per day for 5 weeks appeared normal, while animals receiving about 60,000 IU per kg per day showed abnormal stance with varying degrees of lameness and hind limb immobilization, extensive weakness, increased adrenal, heart, and kidney weights, and elevated serum transaminase (SGOT, SGPT) (115). These results were confirmed using Yorkshire-Hampshire crossbred pigs, 4 to 5 weeks old (116). Normal growth and minimal osseous lesions were found after 5 weeks at the 20,000 IU per kg level, while at the 60,000 IU per kg level, severe lesions occurred in both endochondral and intramembranous bone. Long bones were decreased in length and width with greatest tissue loss in the epiphysis due to lysis of chondroid matrix. Later work in the same laboratory (117) with the same variety of pigs quantitated the osteoblastic response to excess dietary vitamin A. Calcified
tranverse diaphyseal sections of radii from pigs receiving 33,000 IU per kg per day for 5 weeks showed significantly decreased growth rate; appositional bone growth in these pigs was 0.059 mm compared to 0.127 mm in control pigs receiving 350 IU per kg per day. Radii from hypervitaminotic-A pigs had fewer and thinner osteoid seams than radii from control pigs. The investigators suggested that porcine vitamin A toxicosis leads to decreased osteogenesis secondary to decreased osteoblastic activity. Gorgacz et al. (118) found a level of 26,000 IU per kg of retinyl acetate to be mildly hypervitaminotic when fed to Holstein calves, while a level of about 58,000 IU per kg produced depressed growth, hyperhidrosis, alopecia, hyperemia of oral, nasal, and anal mucosae, lameness, and abnormal stance and horn growth.

In an attempt to shed light on the mechanism of hyper-vitamin A toxicity, Mallia et al. (119) fed Holtzman strain rats about 100,000 IU of vitamin A as retinyl acetate daily (about 400,000 IU per kg body weight) for 23 days, together with trace amounts of \(^{3}H\)-retinyl acetate daily. In the blood of these animals 84 percent of the administered vitamin A was found in the lipoprotein fraction unbound to RBP, compared with 18 percent in animals receiving about 1,600 IU as retinyl acetate per kg. In the animals receiving the high doses of vitamin A, substantial and significant decreases in the level of serum RBP occurred, leading the investigators to suggest the possibility that excess vitamin A leads to decreased rate of RBP synthesis in, and its secretion from, the liver. The investigators concluded that serum lipoproteins play an important role in the transport of vitamin A during hypervitaminosis and that toxic manifestations of vitamin A overdosing appear when vitamin A circulates in the plasma in a form not bound to retinol binding protein. They speculated that the nonspecific and unregulated delivery of vitamin A to the tissues in this manner may lead to vitamin A toxicity. Data consistent with this interpretation have been obtained in limited clinical studies of vitamin A toxicity (120).

In summary, it appears that the lowest reported adverse effect level in experimental animals is about 30,000 IU per kg per day for periods of 3 to 5 weeks (112,115-118).

**Chronic toxicity - man**

Prolonged administration of excessive amounts of vitamin A in man can lead to a variety of signs and symptoms characteristic of hypervitaminosis A. In an early report, in 1944, a child receiving 240,000 IU of vitamin A daily (as halibut liver oil) between the ages of 3 months and 3 years (dose level about 50,000 IU per kg at the start, assuming a 5 kg child), showed enlarged liver and spleen, hypoplastic anemia, leucopenia, precocious skeletal development, and clubbing of the fingers. After administration of halibut liver oil was discontinued, most of these manifestations disappeared (121).
Since 1951 many instances of hypervitaminosis A in adults have been described involving patients who had consumed from 100,000 to 600,000 IU or more of vitamin A daily (about 1,600 to 10,000 IU or more per kg body weight) for periods ranging from several months to 8 years before hospital admission (77,122-131). Complaints varied in severity but consistently included headache, hair loss, joint and bone pain, anorexia, nausea, skin rash, irritability, fatigue, and depression. The patients occasionally experienced diplopia, hepatomegaly, tachycardia, hypercalcemia, and edema of the extremities, and infrequently showed bone changes or central nervous system signs or symptoms. In one 18-year-old female who had consumed 200,000 IU of vitamin A daily (about 3,300 IU per kg) for about 2 years, measurement of rib bone biopsy specimens showed the presence of osteocyte lacunae of greater than usual size and bone resorption approximately 6 times greater than in rib specimens taken from 6 normal persons of similar age (130). One 54-year-old male who had consumed about 800 IU of vitamin A per kg body weight daily for 3 years exhibited only central nervous system symptoms or signs, and liver enlargement. Vitamin A blood level in this patient, 69 µg per 100 ml, was within the normal range (125).

A study of 16 females and 1 male (ages 14 to 62) suspected of suffering from hypervitaminosis A revealed that vitamin A intoxication characterized by skin dryness, hair loss, weakness, joint pain, anorexia and headache, occurred within 2 months at daily doses of 200,000 to 275,000 IU (about 3,300 to 4,600 IU per kg). The smallest dose leading to intoxication was found to be about 700 IU per kg daily taken for 8 years (132).

Körner and Völlm (133) assembled and reviewed data on 132 cases of all ages of presumed chronic hypervitaminosis A. About 75 percent of these cases were genuinely hypervitaminotic and were about equally divided between those who had medicated themselves and those for whom the vitamin had been prescribed. As liver storage capacity was exceeded (about 10-fold normal level), signs of hypervitaminosis A appeared. With comparable doses, symptoms of hypervitaminosis A appeared earlier when the vitamin was given in the emulsified rather than in an oily form, because of its better absorbability in the former state. Liver concentration of vitamin A was highly correlated with daily intake per kg body weight and its duration in these cases. At a daily intake of 5,000 IU per kg of vitamin A in oily solution, hypervitaminosis A symptoms began to appear in about 3.5 years. In contrast, equal doses of aqueous emulsions produced symptoms within 7 months. The investigators suggest, as a rule of thumb, the daily dose per kg body weight, multiplied by the duration in days, should not exceed 1 million.

Hawkins and Burlon (134) have suggested that 50,000 IU per day (about 800 IU per kg) for not more than 2 months is the highest advisable dose of vitamin A for treatment of diseases of skin and mucous membranes.
Oliver (126) summarized 36 cases of vitamin A intoxication which had been reported by 1958. In infants who had received daily vitamin A overdoses for periods of several months, all showed bone changes on X-ray examination. Many showed enlargement of the liver, skin lesions, loose hair, and fissured lips. Of the patients receiving only moderate overdoses of vitamin A (135-138), the smallest dose found to elicit these signs, which appeared at age 28 months, was about 3,000 IU per kg body weight daily, consumed over a period of 17 months beginning at age 12 months (136). Seven infants or young children receiving 75,000 to 500,000 IU (7,500 to 50,000 IU per kg assuming a 10 kg child) of vitamin A daily for 6 to 15 months exhibited pruritis, hyperirritability, swelling, and pain of the extremities accompanied by hyperostosis. The minimal toxic daily dose was estimated to be 75,000 units (about 7,500 IU per kg) (139).

Pereira and Begum (140) fed 23 children (age 2 to 6 years) a daily dose of 50 mg retinyl palmitate in peanut oil (about 9,000 IU per kg for a 10 kg child) for 18 weeks with no apparent adverse effects.

Siegel and Spackman (141) found hypervitaminosis A in two siblings. The 12-month-old female had been receiving 25,000 IU vitamin A daily for 9 months (about 3,200 IU per kg) and was hospitalized for vomiting, irritability, exfoliative dermatitis, palpable liver, bulging anterior fontanel, and an elevated serum vitamin A level. The 30-month-old male who had been receiving 57,000 IU vitamin A daily for 1 year (about 4,000 IU per kg) exhibited anorexia, lethargy, pain in the legs, enlarged head, slightly enlarged liver and spleen, and alopecia. Both recovered on discontinuance of vitamin A supplementation.

Persson et al. (142) reported 5 cases of chronic vitamin A intoxication among infants 3 to 5.5 months old receiving 18,500 to 60,000 IU daily (about 2,000 to 7,000 IU per kg body weight) of an aqueous preparation of the vitamin for 1 to 3 months. Characteristic features of the intoxication were elevated fasting blood levels of vitamin A, anorexia, irritability, increased intracranial pressure, skin desquamation, occipital edema, pronounced craniotubes, reduction in skeletal calcium content, and cup-shaped deformations of the widened metaphyses, which were sharply demarcated from the epiphyseal cartilages in the wrists and ankles. Withdrawal of excess dietary vitamin A led to cure in all cases with return to normal skeletal growth within 3 to 4.5 years. As a continuation of this study Tunell et al. (143) fed 2,500 IU of aqueous vitamin A daily to 7 infants beginning at age 1 to 2 weeks for 3 to 5 months, and 7,500 IU daily to a similar group of infants for the same period. No symptoms of vitamin A intoxication occurred at either dosage but serum vitamin A level was significantly higher in the infants receiving the higher dosage. The investigators concluded that although the risk of hypervitaminosis A cannot be excluded at a daily dose level of 7,500 IU (about
900 IU per kg), it has not been demonstrated that chronic vitamin A toxicity occurs at that level. Moore (144) has suggested that hypervitaminosis A represents an exaggeration of the vitamin's normal actions.

The least adverse effect intake in human subjects appears to be in the range of 700 to 3,000 IU per kg per day for periods of several months with most estimates falling closer to the top of the range. Dosages employed in the human studies reported are difficult to equate since it is not always clear whether water miscible or oil soluble vitamin A preparations were used; it is recognized that the former are the more readily absorbed and more capable of eliciting toxic signs at lower dosage levels. Nevertheless, daily intakes of 700 to 3,000 IU per kg would be difficult to achieve from usual dietary sources where mean daily vitamin A intakes, as indicated previously, are of the order of 80 IU per kg for adults to 300 for infants. However, the Committee on Drugs and the Committee on Nutrition of the American Academy of Pediatrics, aware that daily intake can be increased by use of over-the-counter vitamin preparations, indicated that daily doses of 25,000 IU or more of vitamin A for extended periods pose a risk and should not be used except in severe cases of vitamin A deficiency (145). A daily dose of 25,000 IU would amount to 2,500 IU per kg body weight in a 10 kg child and about 400 IU per kg in an adult. Reacting to this statement and other warnings that hypervitaminosis A could result from imprudent dosing with readily available vitamin preparations, the Food and Drug Administration in 1973 issued regulations restricting to prescription sale, oral preparations containing more than 10,000 IU vitamin A per dosage unit (146). This regulation was revoked in 1978 pursuant to an order by the U.S. Court of Appeals concerning the basis of FDA's regulation (147).

Effects on reproduction

March et al. (148) found that by feeding laying chickens 410,000 IU vitamin A as the palmitate per kg of diet, rate of egg production, egg size and hatchability were depressed. At a level of 210,000 IU per kg of diet, egg production, size, and hatchability were essentially normal. Dubiel et al. (149) found single small intramuscular doses (20,000 to 40,000 IU vitamin A per kg body weight) increased volume of ejaculates and sperm survival in male rabbits, while large doses (60,000 to 90,000 IU per kg) decreased the percentage of motile sperms and decreased their survival time. Gellert (150) gave high but non-toxic oral doses of retinyl palmitate (5,000 IU, 3 times per week) to rats for 9 months and observed an inhibition of cyclic ovulatory activity.

Teratogenicity

Congenital malformations were known to occur in the offspring of vitamin A-deficient mothers (144). However, the ability
of a large oral maternal dose of vitamin A to cause congenital anomalies in the rat was first reported in 1953 by Cohan (151). He found that about 175,000 IU per kg daily of an aqueous preparation of natural vitamin A, intubated between the 3rd and 16th days of gestation, reduced the number of litters carried to full term (10 of 100 mated females compared to 44 of 50 in the controls) and the average litter size (7.4 compared to 9.3 in controls). Incidence of congenital defects in 74 offspring of vitamin A-treated animals was 54 percent with zero in the controls. In summarizing the results of more than 100 papers on the teratogenicity of vitamin A published since that time, Shenefelt (152) has indicated that large doses of vitamin A, some many hundredfold the daily requirement in pregnant animals have been shown to produce more than 70 types of malformations in the rat, mouse, hamster, guinea pig, rabbit, dog, pig, and monkey. The type and incidence of malformations depend on dose and stage of pregnancy, and to a lesser extent on species and strain.

The following definitive studies shed light on the influence of doses of vitamin A on the mortality and incidence of teratogenic effects in offspring.

Giroud and Martinet (153) gave retinyl acetate or retinyl palmitate orally to Wistar rats from day 2 to day 14 of gestation in daily doses of 20,000 to 60,000 IU. At the 35,000 IU level (about 235,000 IU per kg body weight assuming 150 g rats) abortions occurred in 90 percent of the animals. Lowering the dose to 20,000 IU (about 135,000 IU per kg) resulted in some resorptions (number not given) and a number (not given) of abnormal embryos showing malformation of the encephalon, anophthalmia, microphthalmia, cleft palate, or cataracts. Internal organs appeared normal. Experiments with much higher doses (about 400,000 IU per kg) given daily for 2 to 3 days during early, middle, and late gestation, showed that the percentage of resorption decreased the later vitamin A was administered during the gestational period, becoming negligible after the 12th day. More anomalies occurred when vitamin A was given during early pregnancy; when given on the 14th to 16th days only cleft palates were observed in the offspring, and on the 18th to 20th days, only cataracts. At comparably high dose levels the same investigators (154) found rabbits to be more sensitive than rats to the abortifacient effects of vitamin A but less sensitive to the teratogenic effects. Murakami and Kameyama (155,156) also showed decreasing frequency of resorptions during the latter stages of pregnancy in mice when injected intraperitoneally with a single 15,000 IU dose (about 750,000 IU per kg) of water miscible vitamin A. Resorption rate was no higher than in controls when injection was made after the 12th day of gestation and substantially fewer offspring showed malformations (20 percent compared with 100 percent in animals receiving vitamin A during first 9 to 10 days of gestation). Marin-Padilla and Ferm (157) reported similar results for golden hamsters intubated with single 20,000 IU doses of vitamin A (about 130,000
IU per kg assuming 150 g hamster) on days 5 through 11 of gestation. There were few resorptions when the vitamin was administered after the 8th day of gestation and malformations did not occur when animals were treated after the 10th day. Robens (158) fed golden hamsters varying amounts of water dispersible retinyl palmitate daily on days 6 to 10 of gestation. About 60 percent of the offspring of those receiving 150,000 IU of the vitamin per kg per day had terata; about 7 percent at 100,000 IU per kg; and about 5 percent at 75,000 IU per kg. Only 0.4 percent of the offspring of control animals had terata.

Giroud and Martinet (159) studied the teratogenicity in mice of relatively small oral doses of vitamin A (2,500 to 12,500 IU per kg body weight) given daily during the 8th to 10th days of gestation. The 6,250 IU per kg dose caused 28 percent abortions, 63 percent deaths or resorptions, and 37 percent malformed fetuses; the 2,500 IU per kg dose caused 10 percent abortions, 8 percent deaths and resorptions, and no malformed fetuses. These investigators commented that their lowest dose was close to that then used in patients receiving vitamin A therapy (2,500 to 3,000 IU per kg body weight). They commented further that while a dose of 2,500 IU per kg did not seem to be teratogenic in mice, such a dose still provoked abortions. Schärer (160), on the other hand, found that mice and rats had to be treated on days 8, 9, and 10 of gestation with at least 150,000 IU of vitamin A palmitate per kg body weight to bring about fetal mortality and malformations of the young.

Nolen (161) has described strain differences among Sprague-Dawley, Charles River, and Wistar female rats given oral doses of aqueous retinyl palmitate at levels of 50,000 or 75,000 IU per day (about 250,000 or 375,000 IU per kg body weight) on days 6 to 15 of gestation. Both Sprague-Dawley and Charles River rats were more sensitive (relative incidence of and pattern of malformations) than the Wistar rats at both dose levels.

Several investigators have reported the effects of large maternal doses of vitamin A on the behavior and learning ability of offspring. All Fischer rat offspring survived maternal doses of 10,000 to 40,000 IU per kg retinyl palmitate, intubated on the 8th, 9th, or 10th days of gestation. At 70 days of age the weights of experimental offspring were the same as the control animals but the vitamin A-exposed animals, particularly those of dams receiving the highest dose, showed impaired learning ability as measured by maze avoidance tests (162). No impaired learning ability but slower response rates have been reported by Hutchings and Gaston (163) for offspring of Wistar rats given 450,000 IU per kg body weight of water soluble vitamin A intragastrically on days 17 and 18 of gestation. Butcher et al. (164) observed impaired learning ability at age 50 days in offspring of Sprague-Dawley rats intubated with 100,000 IU vitamin A per kg body weight on the 8th, 9th, and 10th days of gestation.
The available information concerning the teratogenic potential effects of vitamin A provides some basis for estimating the highest dose at which teratogenic effects do not occur. It has been demonstrated at all hypervitamin-A levels that the stage of pregnancy when the vitamin is administered has a critical bearing on the appearance of teratogenic effects. However, the relatively few experiments in which moderate maternal vitamin A dosages were administered at the stage of pregnancy when teratogenic effects are most likely to occur, indicate that the no-effect level in mice could be as low as 2,500 IU per kg body weight, in hamsters about 75,000 IU per kg, and in rats about 135,000 IU per kg. Thus, species differ in sensitivity. No information is available on the relative sensitivity of man. However, the lowest dose indicated above is more than 25 times greater than adult human intakes of vitamin A from food sources, estimated earlier in this report to approximate 100 IU per kg per day. Some therapeutic dose levels to vitamin A may approach 2,500 IU per kg for short periods of time.

The following papers represent attempts to elucidate possible mechanisms for the production of teratogenic effects in offspring of animals treated during pregnancy with excessive amounts of vitamin A.

Kochhar and Johnson (165) studied cleft palate information in fetuses of black-hooded female rats given about 360,000 IU of vitamin A acetate per kg daily for 3 consecutive days beginning on the 9th or 10th day of pregnancy. More than 80 percent of the embryos developed cleft palate. In many of these embryos, there was a considerably lesser amount of mesenchymal tissue contributed by the maxillary processes to form the palatine shelves than in the control animals. A process of heterotopic chondrogenesis was detected within the preosteoblastic tissues of maxillae and palatine shelves which, in 17-day embryos, had already replaced a major portion of the maxillary bone. Soliman (166) gave pregnant rats 250,000 IU retinyl palmitate per kg body weight during days 8 to 10 of gestation. Approximately three-quarters of their fetuses showed anomalies. Normal control values were not reported. Subcutaneous injection of 0.2 g α-tocopherol on gestation days 8 and 12 to another group of A-hypervitaminotic rats lowered the incidence of fetal anomalies from 76 to 48 percent. As a result of detailed morphological examination of abnormalities occurring in fetal Wistar rats obtained at days 8, 9, 10, and 11 from pregnant animals injected with about 500,000 IU per kg body weight of aqueous retinyl palmitate on the 8th day of gestation, Morriss (167) suggested that malformations originated from a loss of synchrony in the developmental process because of differential toxicity of vitamin A to the three germ layers. Light and electron microscopic studies of the decidual and trophoblastic tissue of the pregnant rat made by the same investigator 5 h after maternal administration of about 500,000 IU vitamin A palmitate per kg body weight, showed membrane damage close to capillaries, vacuolated and
distorted blood cells, and cell debris in the blood space around the embryo. Decreased oxygen-carrying capacity and increased peripheral resistance were suggested as maternal vascular factors complementing the direct embryotoxic effect of vitamin A in the teratogenic mechanism (168). In later studies, Morriss and Steele (169) demonstrated that retinol had a direct teratogenic effect on rat embryos explanted on the 8th day of gestation and cultured in the presence of 0.5 to 20 μg retinol per ml.

A number of experiments with explanted embryos or embryonic tissues in culture appear to confirm the direct teratogenic effects of vitamin A. Degradation of the extracellular matrix of chick limb-bone rudiments occurred in culture solutions containing approximately 5 to 10 IU retinol per ml but not when retinol, complexed with retinol binding protein, was used (170). A concentration of 10 IU retinol per ml interfered with cell movement in cultured mouse limb buds (171) and with cell division and metabolism in cultured chondrocytes from embryonic chick sternum (172). Up to 30 g retinyl acetate (100 IU) per ml of culture solution caused a dose-dependent inhibition of acid mucopolysaccharide synthesis in embryonic chick sternum chondrocytes although collagen synthesis was not inhibited (173). A concentration of 10 IU of retinol per ml of medium caused decreased chondriification and keratinization of metacarpals and abnormal development in forelimb buds of embryos taken on the 10th to 12th day of pregnancy from ICR-JCL mice (174,175). Terashima and Nogami (176) used 35S-sulfate, injected into newborn and 15 to 18 day-old fetuses, to study cartilage metabolism in the extremities of Sprague-Dawley rats whose mothers had received intraperitoneal vitamin A (about 2 million IU per kg body weight) on the 11th day of pregnancy. They observed increased synthesis and degradation of sulfated glycosaminoglycans in the fetal cartilage. March et al. (148) found 1,070 IU retinol, injected into eggs prior to incubation, to depress hatchability markedly whereas 8,000 IU of retinyl palmitate had little effect. Developmental abnormalities were noted in a number of dead embryos from retinol-injected eggs and hemorrhaging appeared to be a frequent cause of embryonic death.

Some case reports have been published suggesting that congenital abnormalities may occur in humans whose mothers have been exposed to excessive amounts of vitamin A during pregnancy. Bernhardt and Dorsey (177) attributed urinary tract malformations in a female infant to the consumption of 25,000 IU of vitamin A (as capsules of fish liver oil) by the mother daily for the first 3 months of pregnancy and 50,000 IU daily from the fourth through the ninth months. These investigators referred to another instance where urinary tract malformations in a human infant were attributed to consumption of 40,000 IU of vitamin A by the mother daily from the sixth to the tenth week of pregnancy. Stånge et al. (178) considered malformations of the central nervous system in a human infant to be related to high doses of vitamin A (150,000 IU
daily during days 19 to 40 of gestation) given the mother as treatment for acne. Gal et al. (179) found higher levels of vitamin A in blood obtained 7 days postpartum from women delivering babies with central nervous system defects than in blood samples from women delivering normal babies. Significance of this finding was considered unclear by these investigators because of the normally wide variation in blood vitamin A levels and the lack of specific information on the blood vitamin A levels during pregnancy. Yeung (180) has shown that young women receiving oral contraceptives have significantly higher mean vitamin A plasma levels than those not using this medication. Elevation of plasma level of the vitamin was not due to variations in vitamin A intake. Yeung indicated that the physiological implication of these findings in subjects receiving oral contraceptives is not clear. However, according to Wild et al. (181) and Larsson-Cohn (182) such elevated levels of plasma vitamin A probably represent no risk to the offspring of women taking oral contraceptives shortly before becoming pregnant. The conclusions of a review of recent studies on the effect of oral contraceptives on human plasma vitamin A agree with this latter view (183).

In a recent comprehensive review of hypervitaminosis A induced teratogenicity, Geelen (183a) has concluded that there is no definitive proof of teratogenic effects of excess vitamin A in the human but results in animals of different species suggest that hypervitaminosis A in pregnancy may have serious consequences for the developing embryo and fetus.

Mutagenicity

Retinyl acetate was found to be without mutagenic activity by in vitro plate and suspension tests both with and without activation by mouse, rat, or monkey tissue homogenates. Concentrations up to 0.25 percent were used in tests with Salmonella typhimurium strains TA-1535, -1537, -1538, -98, and -100, and up to 1.7 percent in tests with Saccharomyces cerevisiae, strain D4 (184).

Carcinogenicity

The Select Committee has found no reports indicating that vitamin A is carcinogenic. However, vitamin A may enhance or inhibit responses to viral or chemical carcinogens, particularly during the preneoplastic phase after initiation but prior to cellular transformation (185,186). Sporn et al. (187) believe there is support for the thesis that deficiency of vitamin A analogs (retinoids) is linked to an increased risk of cancer from chemical carcinogens. They suggest that natural retinoids can prevent the development of epithelial cancer but are limited in their usefulness as chemotherapeutic agents because of inadequate tissue distribution or toxicity. Recent studies of the possible anticarcinogenic effects of vitamin A and its synthetic retinoids, including cis-retinoic acid, may provide a means for overcoming some of these problems (188). The following recent studies are relevant.
Dietary vitamin A deficiency enhances susceptibility to carcinogenesis in the respiratory tract (189), bladder (190), and colon (191) of the rat, as well as the respiratory tract of man (192) after exposure to such carcinogens as polycyclic hydrocarbons, nitrofurans, and aflatoxin. A possible explanation of this enhancement of carcinogenesis is the increased DNA synthetic and mitotic activity known to occur in retinoid-deficient epithelia (188).

Felix et al. (193) found that only 4 of 24 BALB/c male mice receiving 3,100 IU retinyl palmitate (about 150,000 IU per kg) daily in drinking water, developed tumors after challenge with injected cells of a transplantable murine melanoma. All controls developed tumors. Rettura et al. (194) found a slower rate of tumor growth, but not lower tumor incidence, in C3H/HeJ female mice inoculated with C3HBA tumor cells and receiving in the diet about 20,000 to 40,000 IU of retinyl palmitate per kg body weight, compared to controls fed no supplemental vitamin A. The control animals had a mean survival time of 42 to 43 days after inoculation, while mean survival times of treated groups ranged from 54 to 75 days. Seifer and Rettura (195) also found that about 20,000 IU vitamin A per kg of diet increased the resistance of CBA, BALB/c, and C3H mice to the oncogenic virus, MuSV-M, and to the transplantable mammary adenocarcinoma, C3HBA. Smith et al. (196, 197) found no effect of 100 to 2,400 µg of retinyl acetate per week, given intragastrically in divided doses, on incidence of respiratory tumors induced by intratracheal instillations of benzo(a)pyrene in Syrian golden hamsters when the animals were housed in laminar flow cages, but the animals receiving the higher dose of vitamin A experienced a higher incidence of respiratory tract tumors when they were housed conventionally. However, regardless of housing conditions, there was a significant reduction in squamous papillomas of the forestomach in animals receiving the higher dose of vitamin A.

Retinoic acid in concentrations up to 0.3 percent applied topically for 6 to 9 days to dimethylbenzanthracene-induced rabbit ear keratoacanthomas, caused regression of the tumors. Regression was markedly enhanced by concomitant application of fluorouracil (198). Gross and Newberne (199) observed that low doses of vitamin A enhanced, but high doses repressed, incidence of colon carcinoma in rats treated with dimethylylhydrazine. Ong et al. (200) have found a protein in extracts of human carcinomas from lung and breast which binds retinoic acid with high specificity. The binding protein was not detected in normal lung or breast tissue from the same patients.

According to Sporn and associates (187,188,201) retinoids have uniquely important roles in regulating cell differentiation to prevent malignancy development. They believe the enhancement of these intrinsic physiological controls with pharmacological amounts of synthetic retinoids is an attractive approach to
cancer prevention and cite work in their laboratory and those of others in support of his thesis (202-208).

Effects on epithelial tissues

It is well established that adequate vitamin A is essential for the normal differentiation of basal (stem) cells to produce the specific mature cells that characterize the various epithelia of the body (188,209). This knowledge apparently prompted the therapeutic use of large doses (80,000 IU or more daily for several weeks) of vitamin A in certain skin disorders (ichthyosis, acne, chronic atopic dermatitis), a practice that was limited in effectiveness by the concomitant toxic effects of such doses of the vitamin (210). The following papers illustrate effects of hypervitaminosis A on epithelial tissues.

Vedrova and Sapelkina (211) showed that doses of 20 or 40 μg vitamin A daily (about 400 or 800 IU per kg) for 2 to 3 months elicited no skin changes in rats that differed from unsupplemented controls. However, a dose of 80 μg daily (about 1,600 IU per kg) caused thickening of the epidermis, proliferation of cells in the basal epidermal layer, increase in the granular layer, thickened and scaly horny layer, abnormal hair follicles, and disintegrating fat gland cells which were frequently overfilled with sebaceous secretion. Spreca et al. (212) also observed a significant rise in the number of mastocytes with swelling and degranulation in the dermis of rats fed 50,000 to 100,000 IU vitamin A (about 250,000 to 500,000 IU per kg body weight) daily for 10 days.

Pinkus and Hunter (213) studied the stripped off keratin layer of the forearm skin of human volunteers before and after buccal dosing with 2,500 IU vitamin A per kg body weight daily for 30 days. Punch biopsy specimens were also taken of the stripped area. After vitamin A dosing the number of horny cells that could be stripped per unit area decreased, mitosis in the denuded area was decreased in 7 of 10 subjects, and the total number of nucleated cells per unit area was increased. The authors concluded that vitamin A at this level retarded keratinocytic maturation. Sweeney and Hardy (214) found keratinization of skin from the upper lips of 12-day-old embryonic Swiss mice to be suppressed when cultured for 10 days in media containing 5.7 μg retinol per ml. Ciliated and secretory epithelium developed in contrast to squamous stratification and keratinization that occurred in control cultures. Retinyl acetate at 1.56 to 3.12 μg per ml resulted in a 40 percent increase in the cellular RNA content of cultured 1-day-old BALB/c mouse epidermal cells but either increased (6.25 μg per ml) or decreased (0.78 μg per ml) concentrations had less effect (215).
Other biological consequences of hypervitaminosis A

Enzymes - Working in vitro with lysosomes isolated from rat retinas, Dewar et al. (216) found that 45 min incubation with 1.0 to 2.5 μg retinol per mg wet weight increased the release of β-glucuronidase, β-galactosidase and hexosaminidase. Wang et al. (217) noted a similar release of acid phosphatase, β-glucuronidase, deoxyribonuclease; and N-acetyl-β-D-glucosaminidase from female mouse liver lysosomes by vitamin A. Retinol was more effective than retinyl acetate and retinoic acid in affecting acid phosphatase but these forms of vitamin A were equally effective in the release of the other enzymes. Such enzyme release has also been observed in vivo (218). Extensive hemolysis resulted when human erythrocytes were exposed for 20 min to 40 μg of retinol per ml (219).

Rats consuming a diet containing up to 180,000 IU of vitamin A per kg for 1 to 2 weeks showed a 2- to 3-fold reduction in the activity of pancreatic lipase and of amylase, enterokinase, and alkaline phosphatase in the duodenal mucosa, while activity of acid phosphatase remained unchanged. A 2- to 3-fold reduction of the activity of blood serum α-amylase and lipase was also observed (220). Leuts'kii and Baran (221) have also noted a sharp decrease in ATPases in the mucosa of the small intestine and in cell membranes of rats receiving large doses of vitamin A.

Maximum stability of rat liver lysosomes, as measured by the activities of such hydrolytic enzymes as β-glucuronidase, β-hexosaminidase, hyaluronidase, cathepsins, and arylsulfatase, occurred when the rats were fed 100 to 2,000 IU of vitamin A daily (about 1,600 to 33,000 IU per kg body weight); above and below this dosage range, stability was progressively diminished. Since there was no appreciable change in total enzyme activity with variations in vitamin A status and since retinol in vitro had no effect on the enzyme activity released from the lysosomal fraction, the investigators concluded that the action of vitamin A in vivo is a membrane effect (222). Toxic doses of vitamin A were found by Eremina et al. (223) to inhibit the activity of alcohol dehydrogenase in the liver, kidneys, and small intestine of the rat; aldehydoxidase activity in the small intestine increased.

Dileepan et al. (224) fed young male Wistar rats daily for 2 days 30,000 IU of retinyl palmitate (about 300,000 IU per kg). The in vitro incorporation by liver slices of 14C-labeled precursors, such as alanine, bicarbonate, or glycerol, into glucose and glycogen was studied 24 h after the last vitamin A feeding. Amino acid catabolizing enzymes were also studied. Stimulation of hepatic gluconeogenesis in hypervitaminosis A was indicated by increased incorporation of 14C-labeled alanine and bicarbonate into glucose and glycogen. There were marked increases in the activities of hepatic alanine aminotransferase
and ornithine aminotransferase and a decrease in that of trypto-
phan pyrrolase. Activities of liver tyrosine aminotransferase and
serine dehydratase were unaffected.

**Lipid metabolism** - Large doses of retinol (about 70,000
to 500,000 IU per kg body weight) fed daily for 2 to 10 days to
Wistar rats caused significant increases in total liver lipids,
total fatty acids, glycerides, and esterified cholesterol, but
decreased adrenal levels of cholesterol and ascorbate (225–228).

Ahuja and Misra (229) found that feeding male Wistar rats
100,000 IU of vitamin A daily (about 1 million IU per kg body
weight) for 2 days reduced the utilization of randomly labeled 14C-
glucose for fatty acid synthesis in liver and adipose tissue. How-
ever, synthesis of neutral lipids and phospholipids was increased
in the liver and decreased in adipose tissue. Ramachandran et al.
(230) found about 2-fold higher levels of free fatty acids in
plasma and epididymal fat pads of male Wistar rats after feeding
about 375,000 IU retinol per kg per day for 2 days; total lipids
and triglycerides in the liver were also increased. In subsequent
work, the same investigators (231) reported that ingestion by rats
of 30,000 IU of retinol daily (about 375,000 IU per kg body
weight) for 2 days increased the liver incorporation of palmitate-
1-14C into triglycerides but not into phospholipids. Oxidation of
palmitate-1-14C to CO2 by skeletal muscle was also increased under
these conditions.

Pokrovsky et al. (232) intubated young male Wistar rats
with single doses of 50,000 to 250,000 IU of retinyl palmitate
(about 900,000 to 4.5 million IU per kg) and found significant
dose-dependent lowering of NADPH-dependent and ascorbate-dependent
lipid peroxidation in liver microsomes after 2 to 6 days, suggesting
that under these conditions vitamin A may act as an antioxidant.
Gerber and Erdman (233) have reported that vitamin A, particularly
in the form of retinoic acid, fed for 28 days at levels as low as
about 8,000 IU per kg, induced hypertriglyceridemia in male
Sprague-Dawley rats; this effect was not mediated by the adrenals.

High dietary levels of vitamin A (a minimum of 15,000 IU
per kg feed) have been reported to reduce the severity of athero-
sclerosis in Japanese quail (234) but divergent effects of high
levels (22,000 IU per kg of feed) were observed in different
strains of chickens (235).

**Carbohydrate and protein synthesis** - Rats given large doses
(up to 50,000 IU per animal) of vitamin A daily showed a 50 per-
cent decrease in epithelial proteins (presumably collagens) soluble
in 0.5 M NaCl and a 25 percent decrease in skin proteins soluble
in citrate buffer (236). Ahuja and Misra (237) fed male Wistar
rats 33 mg retinol (about 100,000 IU per kg) daily for 2 days.
Twenty-four hours after the last retinol feeding, leucine-1-14C
was injected intraperitoneally to study its incorporation into liver, plasma, and muscle proteins. The retinol-fed rats showed no significant changes compared with controls in the amount of protein per g of tissue in liver, muscle, or plasma, but plasma urea concentration was approximately doubled; incorporation of leucine-1-14C was significantly reduced in muscle proteins and increased in liver proteins in the retinol fed rats. Retinol feeding under these conditions increased liver and adrenal weights relative to body weight, but decreased the ascorbic acid, cholesterol, and lipid content of the adrenals. Chadaeva and Matusis (238) fed doses of 150 IU or 2,000 IU of vitamin A (about 750 or 10,000 IU per kg body weight assuming 200 g animals) to rats for 2 months and found a 2-fold or greater increase in the sialic acid (the N- and O-acyl derivatives of a deoxynami sugar acid) content of liver mitochondria. The investigators regarded this as significant because of the roles played by vitamin A and sialic acids in the functioning of biomembranes. It now appears to be established that retinyl phosphate and its mannosyl derivative, mannosyl retinyl phosphate (MRP), are synthesized by mammalian membranes in vivo and in vitro (239). Recent evidence supports the concept that MRP is involved in glycoprotein synthesis in a large variety of tissues (240).

Nucleic acids - Young male Wistar rats (25 days old) fed 5,000 to 40,000 IU of vitamin A daily (about 180,000 to 1.5 million IU per kg) for 2 days were studied for the effects on (a) liver protein, (b) RNA and DNA, (c) liver and plasma lipids. At the lower vitamin A doses the level of each of these substances was equal to, or slightly greater than the controls while at the highest vitamin A dose each was lower than controls (241). The same investigators (242) also reported that liver content of RNA and its synthesis from orotic acid-6-14C in the liver, as well as its nuclear and mitochondrial fractions, were not affected by feeding 180,000 IU vitamin A per kg but were reduced in rats fed about 720,000 to 1.5 million IU per kg of vitamin A for 2 days.

Blood - Daily intraperitoneal injection of about 70,000 IU retinyl palmitate per kg body weight for 3 months caused lymphomonocytosis and marked changes in organs of the lymphomyeloid complex in male guinea pigs (243). Soliman (244) reported that the blood of pregnant rats receiving 250,000 IU vitamin A palmitate per kg body weight during days 8 to 10 of gestation showed a significant decrease in hemoglobin, hematocrit, erythrocyte and thrombocyte counts, and in the concentration of various coagulation factors.

Clinical study of several patients (ages 7 to 46) has indicated that hypercalcemia may result from vitamin A overdosing ranging from 2,000 to 4,000 IU per kg per day for from 6 months to 7 years, making it advisable to consider possible hypervitaminosis A in the differential diagnosis of hypercalcemia (245,246).
Cerebrospinal pressure - Maddux et al. (247) investigated the effect of oral vitamin A on cerebrospinal fluid pressure and brain water in Sprague-Dawley rats. Immature rats given 7.5 mg retinol per day (about 300,000 IU per kg) for 3 to 8 days showed a 73 percent drop in cerebrospinal fluid pressure after 3 to 5 days and a 93 percent drop after 6 to 8 days. Brain volume increased 2 percent.

Immune reactions - A number of studies point to the important involvement of vitamin A in immune responses. Krishnan et al. (248) observed marked atrophy of the thymus and spleen in vitamin A-deficient rats. Cohen and Cohen (249) also found that daily intraperitoneal injections of retinyl palmitate in the mouse markedly increased the normal number of antibody-forming cells generated in the spleen in response to immunization with sheep red blood cells.

Dresser (250) found vitamin A to act as an adjuvant in converting a non-immunogenic antigen (bovine gamma G protein) to an immunogenic form in mice. It was speculated that vitamin A's "adjuvanticity" may result from its damaging effect on lysosomal membranes, thus stimulating cell division. It was thought that stimulation of cell division at the time when antigen is available in the cell may lead to induction of immunity.

Jurin and Tannock (251) investigated the influence of vitamin A (150,000 to 250,000 IU per kg body weight as retinyl palmitate Aquasol® injected daily intraperitoneally on 5 consecutive days) in mice on their immunological response as measured by the titre of haemagglutinin 10 to 15 days after sensitization with sheep red blood cells, or by the time required to reject male mouse skin grafts by isologous female recipients. Daily vitamin A injections for 5 days preceding or following sensitization with sheep red blood cells led to a large increase in the production of haemagglutinin antibodies. After similar vitamin A treatment there was a significantly reduced mean rejection time of male skin grafts.

Mice given 4 consecutive daily intraperitoneal injections of 3,000 IU of water-miscible retinyl palmitate (about 135,000 IU per kg body weight) and subsequently injected intraperitoneally with gram-negative Pseudomonas aeruginosa, or gram-positive Listeria monocytogenes, or the fungus, Candida albicans, showed a significant increase in survival as compared to controls. Since vitamin A did not affect in vitro growth of the three microorganisms it was concluded that the vitamin induces non-specific resistance to infection in mice (252). Vitamin A has also been shown to have profound influence on the course of infection in rats by the nematode Angiostrongylus cantonensis (253) and by the malarial parasite Plasmodium berghei (254).
Uhr et al. (255) found that acute hypervitaminosis A (about 600,000 IU per kg) in guinea pigs can substantially suppress delayed-type hypersensitivity and inflammatory response to intradermal injection of diphtheria toxin, and suggested that the effect was a consequence of the action of vitamin A on lysosomes. Hypervitaminosis A in guinea pigs has no effect on the clearance of bacteriophage φ174 from the circulation or on antibody formation to the phage. Acute hypervitaminosis A, characterized by weight loss, weakness, hair loss, and decreased muscle tone within 24 h after administration, was produced by oral administration of 160 mg (about 500,000 IU) of retinoic acid per kg body weight.

Lucy et al. (256), studying the possible role of intracellular proteinases in the degradation of cartilage matrix in chick limb bone rudiments in culture, found that normal chondrocytes contain an enzyme(s) capable of producing effects on cartilage matrix that closely resemble those produced by excess of vitamin A. They suggested that further study is needed to establish whether there is a relationship between the enzyme(s) and vitamin A in this report.

In studies of vitamin A-deficient children, Bhaskaram and Reddy (257) found T-lymphocytes to be decreased but cell function to be unchanged, leading them to suggest that vitamin A may act as an adjuvant to promote lymphocyte proliferation.
V. OPINION

Vitamin A is an essential nutrient for man and other animals. Deficiency of vitamin A causes at least four physiologically distinct and clinically recognized states: loss of night vision; defects in bone growth; defects in reproduction; and defects in the growth and differentiation of epithelial tissues. The recommended dietary allowance of vitamin A for adults is about 3,300 International Units (IU) daily. A daily intake of 3,300 IU would amount to about 50 IU per kg for an adult.

Dietary vitamin A activity is supplied by animal products (preformed vitamin A), plant products (provitamin A, such as carotene), and by the addition of vitamin A (retinol) and/or its esters (retinyl acetate and retinyl palmitate) to fortify certain foods. Mean daily intake of vitamin A from all food sources (excluding vitamin preparations) was approximately 5,000 IU in 1971 to 1974. However, some 47 percent of young adults were found to consume no more than 3,500 IU daily. Nevertheless, there is no clear evidence that vitamin A nutriment is a problem of public health significance in the United States. Per capita daily intake of vitamin A used for the fortification of foods is assumed to be about 800 IU (about 13 IU per kg body weight in adults). However, accurate data on the amounts actually added are not available. The Select Committee believes such data should be obtained.

Signs of hypervitaminosis A in laboratory animals include abnormal bone development and fractures, exophthalmos, intramuscular hemorrhages, alopecia, reduced rate of growth or weight loss, adrenal hypertrophy, and at highly toxic dose levels, death. The lowest reported adverse effect level in experimental animals appears to be in the range 25,000 to 60,000 IU per kg per day for periods of 3 to 5 weeks. In man, where expressed feelings of pain or illness on the part of the patient provide an early indication of adverse effects, the symptoms and signs of hypervitaminosis A vary in severity with the dose level, and include skin dryness, anorexia, headache, weakness, hair loss, joint pain, vomiting, irritability, enlarged liver and spleen, and bulging fontanel and increased intracranial pressure in babies. The lowest reported adverse effect level in man appears to lie in the range 700 to 1,000 IU per kg per day, if continued for periods of several months.

With excessive maternal doses of vitamin A, abortions, resorptions, and a large variety of teratogenic effects can be consistently produced in experimental animals, the effectiveness of vitamin A in this regard being greater when administered in the early stages of pregnancy. Administration of a range of doses of vitamin A at the stage of pregnancy when teratogenic effects are most likely to occur, indicate that the highest no-effect level in
mice, rats, and hamsters lies in the range 2,500 to 100,000 IU per kg per day. Species differ in sensitivity; the small amount of information available with respect to the relative sensitivity of man indicates that maternal doses of the order of 700 to 800 IU vitamin A per kg daily for most of gestation may lead to abnormalities of the urinary tract in offspring. It is to be noted that congenital malformations also occur in offspring of vitamin A-deficient mothers.

Vitamin A has been found to exhibit no mutagenic activity in in vitro tests. There is no evidence that it is carcinogenic.

The lowest doses of vitamin A that produce toxic manifestations in animals and humans are manyfold greater than the daily doses human adults receive from food consumed, only a small portion of which represents vitamin A or its esters that are added to food. Nevertheless, this margin of safety may be compromised by the total intake of vitamin A from all sources.

Based on the foregoing considerations, the Select Committee concludes that:

There is no evidence in the available information on vitamin A, vitamin A acetate, and vitamin A palmitate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.
VI. REFERENCES CITED


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Report submitted by:

March 18, 1980

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances
PUBLIC HEARING ON VITAMIN A, VITAMIN A ACETATE, AND VITAMIN A PALMITATE HELD NOVEMBER 19, 1979*

The following individuals made presentations:


A request to make an oral presentation also was received from R. Ullman, Attorney for National Nutritional Foods Association, 7727 S. Painter Avenue, Whittier, California. However, Mr. Ullman was unable to be present and submitted a written statement on November 23, 1979. This statement is not a part of the hearing transcript but is available from the Hearing Clerk, Food and Drug Administration, Washington, D.C.
