8. Summary of Data

8.1 Chemistry, occurrence and human exposure

The components of vitamin A—retinol and retinyl esters—are lipophilic compounds that are inherently unstable, being sensitive to light, heat and oxygen. In the presence of light, their all-*trans*-tetraene double-bond systems can isomerize to provide a mixture of *trans* and *cis* isomers. Heat also affects double-bond stability and reactivity, and oxygen causes the formation of oxygenated species. Being hydrophobic, retinol and its esters (such as the acetate and palmitate) are insoluble in water and plasma unless associated with proteins.

Vitamin A is metabolically derived from carotenoids having at least one terminus consisting of a cyclohexenyl ring. β-Carotene, with two such terminal rings, is the most active precursor. Vitamin A activity is reduced by minor structural variations in retinol structure, such as double-bond isomerization or introduction of another ring double bond at the 3-position, but is eliminated by significant changes, such as double-bond saturation. Esterification does not reduce activity unless intestinal absorption is impaired. Retinal has activity similar to that of retinol because it is readily reduced; however, while active in regulating cell differentiation, retinoic acid is inactive in supporting vision and reproduction because it cannot be converted back to retinol or retinal.

Human intake of vitamin A is mainly in the form of the provitamin carotenoids and retinyl esters, which are found most abundantly in foods of plant tissue and animal origin, respectively. These forms are converted to retinol in the body. The average recommended daily intake of vitamin A is approximately 0.6–1.0 mg retinol for men and 0.5–0.8 mg retinol for women. However, in treatment of disorders related to vitamin A deficiency such as xerophthalmia, single doses are much higher. On the worldwide scale, vitamin A deficiency has been identified as a severe public health problem.

Retinyl esters, particularly the palmitate and acetate, are found in vitamin A supplements (as a micronutrient) and fortified foods, as well as in cosmetics used to enhance a youthful appearance, based on the efficacy shown by retinoic acids against adverse dermatological conditions. Carotenoids are also used in nutritional supplements and fortified foods. Supplemental retinyl esters and carotenoids have been investigated in trials for cancer prevention.

Measurement of plasma retinol is most commonly used to assess vitamin A status. However, a relative dose–response test probably gives more accurate information on total concentrations of vitamin A in the body. High-performance liquid chromatography is the method of choice for these and other analyses of vitamin A in biological samples.

8.2 Metabolism and kinetic properties in humans and animals

Retinyl esters are hydrolysed in the intestine to retinol, which appears to be absorbed by binding to a mucosal cell transporter. Many yellow, orange, red and green fruits and vegetables, as well as sea plants and fishes, contain carotenoids, some of which serve as precursors of vitamin A. After being incorporated into lipid micelles and absorbed by intestinal cells, provitamin A carotenoids are primarily cleaved in the centre to form one or two molecules of retinal. Retinal is then largely reduced to retinol. Thereafter, the metabolism of vitamin A derived either from retinyl esters or from provitamin A carotenoids follows the same pathway.

Retinol within intestinal cells is esterified, incorporated into chylomicra and transported via the lymph into the general circulation. A portion of the triglyceride fraction of chylomicra is hydrolysed by lipoprotein lipase, producing much smaller chylomicron remnants that contain retinyl esters, some retinol, and carotenoids. These remnants are removed from the circulation by receptor-mediated uptake, primarily by the liver but also by adipose tissue, bone marrow and other tissues.

Within the liver as well as other organs, stellate cells are the major storage sites for retinyl esters. Retinol is released from the liver primarily as a 1:1 complex with retinol-binding protein (RBP), which further combines with transthyretin. The concentration of holo-RBP

in the plasma is homeostatically controlled over a wide range of total body reserves of vitamin A. The uptake of retinol by peripheral tissues may occur by interaction of unbound retinol with membranes and possibly in some tissues by formation of a complex with a cell-surface receptor for holo-RBP.

Within cells, retinol may be oxidized to retinal and then to retinoic acid, esterified with long-chain fatty acids, oxidized at other positions, or conjugated with glucuronic acid. Most of these enzymatic transformations occur when retinoids are complexed with cellular retinoid-binding proteins. All forms of vitamin A and retinoic acid may also undergo double-bond isomerization. Retinol and retinoic acid may also be degraded by stepwise oxidative cleavage of the polyene chain.

Vitamin A is highly conserved in humans and animals by recycling, in which vitamin A released from the liver is taken up by peripheral tissues and then returned to the liver. The kinetics of this rapid and extensive process have been carefully studied in both humans and animals. The irreversible loss of vitamin A from the body is directly related to the total body stores.

Vitamin A functions in vision as 11-*cis*-retinal, in cell differentiation as all-*trans*- and 9-*cis*-retinoic acids; in the immune response, probably primarily as retinoic acid; and in embryonic development both as retinol and as retinoic acid. Retinol and its metabolites serve as ligands for different functional proteins, with opsins in the eye and with the nuclear receptors RAR and RXR in developing and differentiating cells. The interactions of retinoids with these nuclear receptors seem to be most closely associated with their normal functions, as well as with possible events leading to unregulated cellular proliferation.

Most of the biochemical and molecular details regarding vitamin A physiology have been obtained through study of animal models. Much of this information has come from studies in the rat, a model which, with respect to basic transformations in vitamin A physiology, closely resembles man. However, studies of vitamin A transport and metabolism are increasingly being carried out in the mouse model, primarily because of the development of transgenic and knockout technologies and the increasing availability of suitable transgenic mouse strains. Although less frequently reported, vitamin A transport and metabolism have also been studied in many other species, including most commonly rabbits, pigs, ferrets and chickens. Studies of these species have provided information which is directly relevant to the human situation. The validity of data obtained from animal models for describing the human situation must necessarily depend on the physiological or medical context of the study.

In dosing studies on humans, concentrations of metabolic intermediates in plasma and tissues are markedly affected by the vitamin A formulation employed, inasmuch as the nature of the matrix or carrier, rate of release of vitamin A, and the types of solubilizers and stabilizers used vary. In addition, the patterns of metabolites of vitamin A differ markedly between species. Thus, metabolic transformations are qualitatively similar but quantitatively different among species.

8.3 Cancer-preventive effects

8.3.1 Human studies

There is no evidence from human studies that vitamin A has a generalized cancer-preventive effect. Observational studies have generally been based on estimates of preformed vitamin A in the diet, with some information from older studies that reported only total vitamin A; a small number of studies related use of vitamin A supplements to cancer risk. Measurements of serum retinol are relatively uninformative in well nourished populations due to the strong homeostatic controls that modulate plasma levels of retinol. Intervention studies have been conducted with vitamin A doses ranging from approximately 50 to 250% of typical total vitamin A dietary intakes, except for one high-dose study, although the period of supplementation has not extended beyond five years, and duration of follow-up has been limited. Summarized below are the results of studies on vitamin A in relation to specific cancers.

(a) Lung cancer

No association has been found between dietary intake of preformed vitamin A and risk of lung cancer in many observational studies. Data from a large randomized placebo-controlled trial among North American smokers and asbestos-exposed workers suggested, if anything, an adverse effect on lung cancer incidence of a combination of retinol with β-carotene. In a randomized, placebo-controlled trial of a combination of β-carotene and retinol among former asbestos-exposed workers, no significant reduction in sputum atypia was observed. In one trial in treated lung cancer patients, supplementation with high-dose retinol was associated with a reduction in second primary lung cancers.

(b) Mesothelioma

The risk of mesothelioma was reduced in an intervention trial among Australian asbestos miners given retinol, as compared with those given β-carotene. Mesothelioma risk was not affected by retinol in combination with β-carotene in a North American trial.

(c) Upper aerodigestive tract

Case–control studies have suggested either a modest direct association with high dietary intake of retinol or no association. Two Chinese intervention trials did not show a beneficial effect on oesophageal cancer when retinol was given in addition to zinc or a multivitamin preparation. There was a statistically significant effect on head and neck cancers in a trial in which retinol was given in combination with β-carotene to heavy smokers and asbestos-exposed workers. Leukoplakia of the mouth shows a marked positive phenotypic response to preformed vitamin A, but the original cellular phenotype returns in a considerable proportion of cases after cessation of treatment. Whether treatment with preformed vitamin A reduces or delays the progression of these lesions to carcinoma is not known. This work was performed in populations that may have been vitamin A-deficient, the series were small and original lesions could recur after treatment. Its meaning remains unclear.

(d) Gastric cancer

No association has been found between dietary intake of retinol and risk of gastric cancer in many observational studies. No beneficial effect was detected in two intervention studies in China, where retinol was given in addition to either zinc or a multivitamin preparation.

(e) Colorectal cancer

No association between dietary intake of retinol and risk of colorectal cancer has been found in many observational studies, nor in the one available intervention study.

(f) Skin cancer

No association has been found between dietary intake of retinol and risk of skin cancer in a small number of observational studies. An intervention study in the United States showed no beneficial effect of retinol as compared to placebo on the incidence of basal-cell carcinoma. With respect to squamous-cell carcinoma of the skin, a risk reduction was found in relatively moderate-risk individuals, but not in high-risk subjects.

(g) Breast cancer

No association between dietary intake of retinol and risk of breast cancer has been found in many observational studies, mainly of postmenopausal women, nor was a significant association found in one intervention trial. There is a lack of information to indicate presence or absence of effects in premenopausal women.

(h) Prostate cancer

No consistent association has been found between dietary intake of retinol and risk of prostate cancer in many observational studies, nor in the one available intervention study.

(i) Bladder cancer

No association has been found between dietary intake of retinol and risk of bladder cancer in many observational studies and in the one available intervention study.

(j) Cervical cancer

No association has been found between dietary intake of retinol and risk of cervical dysplasia or

invasive cervical cancer in many observational studies.

8.3.2 Experimental studies

(a) Lung

The chemopreventive effects of retinyl esters on lung carcinogenesis have been studied in rats and hamsters. In one study in rats, retinyl acetate caused a dose-dependent inhibition of lung carcinogenesis. Of three studies in Syrian golden hamsters, one was inconclusive and two showed no protective effect.

(b) Mammary gland

The preventive effects of retinyl esters on mammary carcinogenesis were studied in mice and rats. Of two studies conducted in mice, one showed no protective effect, whereas the other study showed enhanced tumour development. Nine studies were performed in rats using different chemical carcinogens. In eight of these, retinyl acetate protected against mammary carcinogenesis, but in one study the result was inconclusive. The protective effect was enhanced when retinyl acetate treatment was combined with other agents such as selenium or butylated hydroxytoluene or with ovariectomy.

(c) Urinary bladder

The preventive efficacy of retinyl esters was assessed in three rat studies. In one, retinyl acetate protected against bladder carcinogenesis, whereas in two, retinyl palmitate was not protective.

(d) Skin

The preventive effects of retinyl esters on mouse skin carcinogenesis were studied in five experiments. Papilloma development induced by chemical carcinogens was inhibited by retinyl palmitate in two studies, while skin carcinoma development was not affected in another study. In two studies on skin tumour induction by ultraviolet irradiation, treatment was ineffective or inconclusive. In a third study, skin carcinoma induction by ultraviolet radiation was enhanced by retinol.

(e) Other organs

In studies of tumour development in the oesophagus, colon and thyroid, retinyl ester treatment either was ineffective or gave equivocal results. In some organs, including forestomach, colon and bladder, vitamin A deficiency led to increased susceptibility to chemical carcinogenesis.

(f) In-vitro models

In vitro, retinol and retinyl acetate were found to inhibit the proliferation and to modulate the differentiation of a large number of untransformed, transformed and malignant rodent and human cells derived from many different histological types of tissue. Growth inhibition was dose- and time-dependent and reversible. Studies with keratinocytes and tracheobronchial epithelial cells indicated that retinyl acetate can modulate cell proliferation, suppress squamous cell differentiation and enhance mucus cell differentiation. Retinol may reduce cell proliferation by arresting the cell cycle. Similarly, the anchorage-independent growth of human fibroblasts induced by growth factors was inhibited by retinol. In contrast, the response of haematopoietic stem cells to certain growth factors was potentiated by retinol or retinyl acetate.

The effects of retinol and/or some of its metabolites on cell proliferation and differentiation are thought to be mediated by interaction with nuclear retinoic acid receptors, which are members of the steroid thyroid hormone receptor superfamily that are *trans*-acting modulators of gene transcription.

(g) Inhibition of genetic and related effects

Vitamin A and its natural derivatives were evaluated in short-term tests *in vitro* and *in vivo* as modulators of genetic and related effects induced by over 50 physical and chemical agents.

In most but not all studies, vitamin A did not affect the genotoxicity of direct-acting compounds. With genotoxic agents that need to be activated metabolically by cells, vitamin A showed consistent protective effects towards mycotoxins, such as aflatoxin B_1,

heterocyclic amines isolated from food pyrolysis products, and nitrosamines present in tobacco smoke and polluted air. The results were inconsistent or equivocal when vitamin A was challenged with other classes of procarcinogens, including polycyclic aromatic hydrocarbons, aromatic amines and some complex mixtures.

8.3.3 Mechanisms of cancer-prevention

Vitamin A may prevent or delay carcinogenesis at both the initiation and promotion steps. However, the mechanisms through which these effects may be exerted have not been fully elucidated. Although retinol can activate the nuclear retinoid receptors either directly or through some of its metabolites, information on which retinoid-regulated genes are the proximal mediators of the above effects of retinol is scarce. Plausible mechanisms based on findings in cultured cells and animal models include modulation of cell properties (cell proliferation, differentiation, communication, adhesion, migration and invasion) or host properties (immune response, angiogenesis). Analysis of these mechanisms in the context of a chemoprevention trial is required for validation of their relevance.

8.4 Other beneficial effects

Vitamin A relieves the symptoms of vitamin A deficiency, including night-blindness and mild cases of xerophthalmia, whereas more severe cases are irreversible. In populations in which clinically apparent vitamin A deficiency is common, a number of intervention trials have shown that vitamin A supplementation reduces child mortality and morbidity. In relatively well nourished populations, evidence of benefit with vitamin A supplement use is inconsistent. Conditions such as arthritis and many infectious diseases have not been consistently improved by vitamin A supplements. These conditions may often involve changes in vitamin A distribution rather than overt deficiency. Psoriasis and other skin conditions may improve with vitamin A treatment, but less toxic analogues of retinol have been effectively used for the last 25 years. Based on results from observational studies and a large intervention study (CARET), retinol together with β-carotene does not seem to be protective against heart disease. In conclusion, preformed vitamin A has not been unambiguously shown to be protective against any conditions other than the direct effects of vitamin A deficiency.

8.5 Carcinogenic effects

8.5.1 Human studies

A large number of observational epidemiological studies on the relation between dietary retinol and cancer risk have not shown consistent evidence of increased cancer rates. Two trials of vitamin A and primary skin cancer have shown no sign of increased risks of squamous or basal cell carcinoma. Similarly, two trials in China and one in Australia reported no evidence of increases in overall cancer incidence or mortality. One study (CARET) reported an increased risk of lung cancer among high-risk individuals taking a vitamin A supplement in combination with β-carotene. Findings from another trial (the ATBC study) suggest that this apparent adverse effect may be largely attributable to the β-carotene component of the supplement rather than to retinol.

8.5.2 Experimental animals

Retinyl esters were evaluated for carcinogenicity by long-term oral administration in one study with female rats and in one study with male and female rats.

In the study with female rats, a higher incidence of mammary adenocarcinomas was observed in rats fed diets supplemented with high levels of mixtures of retinyl acetate and retinyl palmitate; no dose–response relationship was observed. In the other study, the incidence of benign phaeochromocytomas increased in both males and females with increasing levels of retinyl acetate. The incidence of malignant phaeochromocytomas was significantly increased in high-dose males.

8.6 Toxic effects

8.6.1 Human studies

Toxicity due to hypervitaminosis A occurs in the skin, the circulation (e.g., plasma proteins), internal organs (e.g., liver), the nervous system, and the musculo-skeletal system. Side-effects of systemic vitamin A administration commonly

encountered in the skin include desquamation, cheilitis, brittle nails, skin rashes and alopecia. Circulatory side-effects may include hypertriglyceridaemia, serum enzyme increases and hypothrombinaemia. Hepatomegaly and palpaple or tender liver have been reported. Viral hepatitis, protein energy malnutrition and pre-existing liver disease may predispose individuals to vitamin A toxicity. Nausea and vomiting, anorexia, diarrhoea, weight loss, fatigue, malaise, lethargy, somnolence, weakness and irritability are also commonly reported.

Headache may occur transiently with supplement doses. More severe reactions include elevated cerebrospinal fluid pressure, cranial hypertension, pseudotumor cerebri, altered vision and papilloedema. Drug interactions may occur in patients combining vitamin A with minocyclin (a synthetic tetracycline). Hypervitaminosis A is known to cause demineralization of bone, periosteal calcification and hypercalcaemia, in all age groups. Bone and joint pain and myalgia are commonly associated symptoms.

There have been few studies of the effects in pregnancy of retinol itself, although teratogenic effects of 13-*cis*-retinoic acid and synthetic retinoids are well documented. Case–control studies and a trial of multivitamin supplementation reported no increase in the risk of malformations associated with increased intake of vitamin A during pregnancy, but one cohort study did observe an association with major defects.

8.6.2 Experimental studies

The toxicities elicited by vitamin A in laboratory animals generally reflect those seen in humans and occur in the skin, the circulation, internal organs, and the musculo-skeletal system. No studies in animals have modelled the human neurological toxicities.

The commonly observed effects of retinoids on the skin of laboratory animals include erythema, epidermal thickening, scaling, loosening of the stratum corneum, increases in transepidermal water loss, alopecia and conjunctivitis. Elevations in rodent serum lipid profiles, enzyme activities and coagulation times have been reported. Hypervitaminosis A causes fatty infiltration of the rodent liver and up-regulates Kupffer cell activity. The results of drug interaction studies in rats and mice indicate that there are species differences in the effects of vitamin A on the rodent liver and that the timing of vitamin A administration may also affect the hepatotoxicity of the drug or chemical. Altered intestinal absorptive processes, gallstones, degeneration of myocardial fibres with associated electrocardiographic changes, fatty changes and haemosiderosis in the spleen, glomerulonephritis and necrotizing nephrosis in the kidney, and testicular atrophy in adult and degenerative testicular changes in weanling rodents have also been described. Demineralization, thinning of the long bones, cortical hyperostosis, periostosis, limping gait, fractures, osteoporotic lesions and premature closure of the epiphyses have been described in numerous animal species.

Retinol, retinal, retinoic acid and retinyl esters gave negative results in the large majority of studies evaluating induction of genetic and related effects in bacteria, yeast, mammalian cultured cells and animal models.

Vitamin A (retinol, retinyl esters) was teratogenic in a number of experimental animal studies. The doses needed to elicit a frank teratogenic response were, however, much higher than those expected in human therapy, prophylaxis or nutrition.

Mice and rats not only need extremely high doses to elicit a teratogenic effect, but also exhibit a very different metabolic pattern as compared to humans. Thus, rats and mice are poor models for the human. In the rabbit, much lower doses are sufficient to induce developmental defects. Also, the endogenous retinoid plasma pattern is very similar to that seen in humans after vitamin A administration.

Highly active retinoids have been found in human plasma after vitamin A exposure (supplementation, liver consumption). Their plasma concentrations and AUC values were in some instances in the same range as those found after administration of embryotoxic doses to rabbits, currently the most appropriate experimental animal model. Thus, a teratogenic risk of supplementation and liver consumption in humans can at present not be excluded on the basis of experimental studies. It is not known if a safe threshold dose or exposure exists with regard to developmental defects.