8. Summary of Data

8.1 Chemistry, occurrence and human exposure

The carotenoids are hydrophobic, lipophilic substances which, after ingestion, are absorbed with other lipids. The state in which the carotenoids occur in the food matrix, e.g. crystalline or not, their concentration, the availability of fat or oil and the presence of bile acids are major factors in determining their bioavailability. About 600 carotenoids have been isolated from natural sources, and some 100 or so of these are likely to be present in the human diet; however, of the carotenoids in serum, only six or seven have been studied in any depth, of which three are provitamin A carotenoids, i.e. β-carotene, α-carotene and β-cryptoxanthin. The main carotenoids that are addressed in this *Handbook* are found widely in fruits and vegetables and products derived from them; small amounts are also found in foods of animal origin such as some fish and crustaceans, egg yolk and dairy products. Some carotenoids, notably β-carotene, are used widely as food colourants and are produced synthetically or from biological sources for this purpose. Supplements of β-carotene, natural and synthetic, are widely available.

Fruits and green vegetables are the main sources of β-carotene and lutein in the human diet. Carrots are rich in β-carotene and are often the main source of α-carotene in temperate climates, depending on strain or variety. Tomatoes and tomato products are rich in lycopene. Yellow maize provides zeaxanthin and β-cryptoxanthin; various fruits such as oranges, peaches, apricots, mangoes and papayas, also contain β-cryptoxanthin. There is no major dietary source of canthaxanthin, although it is found in trout, crustaceans and sometimes in egg yolk after its use as an additive in feed. Canthaxanthin has been marketed in the past as an orally administered 'tanning' agent, but this use has been discontinued in several countries. A balanced diet provides a daily intake of a few milligrams of some of these compounds; the total might range from 4 to 10 mg/day. Much higher intakes can be achieved from certain foods such as carrots, red palm oil, mangos and concentrated tomato products. If supplements are taken, the intake increases accordingly. The daily intake of each of the other carotenoids listed, namely zeaxanthin, β-cryptoxanthin, canthaxanthin and α-carotene, is likely to be 1–5 mg.

In the body, all carotenoids are found in lipid environments, especially fatty tissues and membranes. Their presence in membranes may be important in relation to their biological actions.

The long system of conjugated double bonds that constitutes the light-absorbing chromophore of the carotenoids also makes these molecules rather unstable and very reactive towards oxidizing agents and free radicals. They can have antioxidant or pro-oxidant actions *in vitro*. Although antioxidant activity *in vivo* has not been proven, this has been proposed as a possible mechanism by which carotenoids could protect against cancer and other degenerative diseases.

Routine methods for the qualitative and quantitative analysis of carotenoids in foods and in blood and body tissues are usually based on reverse-phase high-performance liquid chromatography with the use of an in-line photodiode-array detector to generate ultraviolet or visible absorption spectra. Mass spectrometry and co-chromatography with authentic samples are required additionally for proper identification.

8.2 Metabolism and kinetics

8.2.1 Humans

In humans, carotenes and many xanthophylls are absorbed in the small intestine and appear

in lipoproteins of the plasma. Although more than 20 carotenoids are found in plasma, the major components are lycopene, lutein, β-carotene, zeaxanthin, β-cryptoxanthin and α-carotene. The types and amounts of carotenoids in the plasma reflect those in the diet. Plasma carotenoids are taken up by essentially all tissues, the major repositories being the adipose tissue, liver and skin. The pattern of carotenoids in tissues reflects that in the plasma, with few exceptions.

Factors that influence serum carotenoid concentrations and presumably also those of tissues include the dietary intake of carotenoids, the fat content of the diet, the acidic fibre content of the diet, smoking, alcohol intake and food processing. The determinants of absorption and resulting blood and tissue concentrations are not well understood. In general, increased intake of both dietary and supplemental carotenoids leads to higher blood concentrations, but the bioavailability of purified or synthetic preparations is greater than than that of dietary components. Oral supplements of a given carotenoid markedly increase its concentrations in plasma and tissues. In vitamin A-sufficient subjects, carotenoid intake has little effect on the plasma concentrations of retinol.

The only known function of carotenoids in humans is as precursors of vitamin A. Only 50 of the approximately 600 carotenoids in nature, however, serve this role. The major pathway of enzymatic conversion is central cleavage of the carotenoid molecule, although asymmetric cleavage can also occur. Carotenoids can also be oxidized at other positions in the molecule, although such reactions have been little studied.

8.2.2 Experimental models

Distinct interspecies differences exist in the biokinetics of carotenoids and particularly in their intestinal absorption, their transport in plasma and, to a lesser extent, their metabolism in tissues. Most of the common laboratory animals are not suitable models for biokinetics in humans; however, two animal models have been developed to mimic the situation in humans: the ferret and the preruminant calf. Although both have some limitations, studies with these species have provided important information on carotenoid uptake and metabolism. Use of non-human primates has provided promising results, but further evaluation of these models is needed. Other species, such as rats, chicks and pigs, may be considered for investigating specific aspects such as metabolism.

Carotenoids are metabolized differently in different species. The most marked difference is between vertebrates that efficiently absorb intact carotenoids, such as humans, other primates, cows and birds, and those that do not, such as most rodents and pigs. Because of these differences, research in humans is particularly important.

8.3 Cancer-preventive effects

8.3.1 Humans

The results of epidemiological studies, viewed in aggregate, do not support the notion that β-carotene has generalized cancer-preventive effects. The observational data suggesting cancer-preventive effects are most consistent for lung, oral and pharyngeal cancers, the incidences of which tend to be inversely related to β-carotene (or provitamin A carotenoid) intake or blood concentrations. One difficulty in interpreting these findings is that β-carotene may be only a marker of the intake of other beneficial substances in fruits and vegetables or perhaps other lifestyle habits. No clinical trial of β-carotene as a single agent, however, has shown a reduction in the risk for cancer at any specific site, and there is evidence of an increase in the risk for lung cancer among smokers and asbestos workers receiving β-carotene supplements at high doses, which resulted in blood concentrations an average of 10–15 times higher than normal. It is worth noting that the information from clinical trials reflects the first 12 years of intervention, and, at present, there are no data on the possible effects of longer intervention. There is virtually no information on β-carotene supplementation early in the carcinogenic process. Lastly, the doses used in the intervention trials greatly exceeded those consumed in normal diets. There is only limited, inconsistent information on carotenoids other than β-carotene.

Summarized below are the results of studies on β-carotene pertaining to specific cancers. The data for other cancer sites were generally less extensive and not indicative of either protection or harm.

Lung cancer

The most extensive results with regard to β-carotene pertain to cancers of the lung and bronchus. The vast majority of observational studies of dietary intake indicate a decreased risk with higher intake of carotene. There are also extensive, consistent findings that higher blood concentrations of β-carotene are associated with a decreased risk for lung cancer. In general, people with the highest intake or blood concentration have been found to have a 20–50% lower risk than those with the lowest values. In contrast, no clinical trial of β-carotene supplementation has shown a reduction in risk, and two of the three large trials found an increase in lung cancer occurrence among smokers; one also suggested an increase in asbestos workers.

Skin cancer

The results of epidemiological studies show no reduction in the risk for skin cancer associated with β-carotene intake or blood concentrations. One clinical trial indicated no reduction in the risk for basal- or squamous-cell skin cancer after supplementation for up to five years.

Oral and pharyngeal cancers

Some observational studies have shown inverse associations between dietary intake of β-carotene (or carotene), blood carotene concentrations and the risk for oral and pharyngeal cancers. Intervention trials of intermediate markers of oral carcinogenesis (oral leukoplakia or micronuclei) with supplemental β-carotene, alone and in combination with other agents, have shown regression. Many of these trials, however, have methodological limitations.

Oesophageal cancer

Observational studies of dietary intake of provitamin A carotenoids generally suggest inverse associations with the risk for oesophageal cancer. The results of two related intervention trials are available. In one trial, supplemental β-carotene given in combination with vitamin E and selenium had no effect on the rate of mortality from oesophageal cancer. In a related trial in the same population but restricted to persons with oesophageal dysplasia, supplemental β-carotene given with several other micronucrients was also of no benefit in preventing death from oesophageal cancer.

Gastric cancer

Some observational studies of either dietary or blood β-carotene concentrations showed inverse associations with gastric cancer or precancerous gastric lesions. The results of three intervention trials are available. In one trial, supplemental β-carotene given in combination with vitamin E and selenium showed a reduction of borderline significance in the risk for gastric cancer. The population studied was known to have several micronutrient deficiencies, and the relevance of these results for well-nourished populations is unclear. In a related trial in the same population but restricted to persons with oesophageal dysplasia, supplemental β-carotene given with several other micronutrients was of no benefit in preventing mortality from gastric cancer. In a third trial, no reduction in gastric cancer risk was observed.

Colorectal cancer and adenoma

Epidemiological studies show no clear pattern of reduced risk for either invasive cancer or adenoma in relation to β-carotene intake. Two clinical trials showed no reduction in the occurrence of adenoma after supplementation with β-carotene. None of the large trials of β-carotene supplementation suggests a decrease in the occurrence of colorectal cancer.

Cervical cancer

Some observational studies have shown inverse associations between dietary intake of various carotenoids or blood carotenoid concentrations and the risk for cervical neoplasia. One trial of low-dose β-carotene supplementation in women with cervical dysplasia in the Nether-lands gave no evidence of greater regression in this group.

8.3.2 Experimental systems

8.3.2.1 Cancer-preventive activity

β–Carotene

The cancer-preventive efficacy of β-carotene has been assessed in mouse, rat and hamster

models, virus-induced tumour models and inoculated tumour cells.

In models of respiratory-tract carcinogenesis, β-carotene was ineffective in three studies in hamsters, and there were even indications of weak enhancement in two of these studies. In models of lung carcinogenesis in mice, β-carotene was ineffective in two studies.

The effects of β-carotene against skin carcinogenesis induced by various carcinogens were investigated in more than 20 studies in mice; β-carotene was effective in almost all. In the two studies in which it was ineffective, it was administered only before initiation or in the late stages of carcinogenesis. β-Carotene was effective in preventing buccal pouch carcinogenesis in hamsters in about 20 studies. It was ineffective in only one study when also given after tumour development. β-Carotene was also effective in preventing liver carcinogenesis in most of about 20 studies in male rats. In those studies involving administration of *N*-nitrosodiethylamine or 2-acetylaminofluorene, conflicting results were obtained. β-Carotene did not affect the incidence of liver tumours in a strain of mice with a high incidence of spontaneous tumours at this site. The cancer-preventive effects of β-carotene in models of colon carcinogenesis were investigated in one study in mice and in seven studies in rats. Conflicting results were found, and no pattern emerged to explain the differences. β-Carotene prevented pancreatic cancer in two of three studies in rats and in one of two studies in hamsters. No pattern emerged to explain the differences. The preventive effect of β-carotene on gastric carcinogenesis was demonstrated in one study in mice but not in one study in rats. β-Carotene prevented urinary bladder carcinogenesis in one of two studies in mice when given before, during and after carcinogen administration, but not in one study in rats. It was ineffective in models of small intestine carcinogenesis in two studies in rats. It showed some preventive effects against salivary gland carcinogenesis in one of three studies in rats.

In one model of multiorgan carcinogenesis in rats, β-carotene showed a tendency to decrease the incidences of preneoplastic liver foci, colon adenocarconomas and nephroblastomas.

β-Carotene was effective in three studies in models of malignant tumours induced in mice by Moloney murine sarcoma virus. In several studies in mice and rats inoculated with tumour cells, subsequent administration of β-carotene inhibited tumour growth, enhanced survival and in some cases resulted in tumour regression.

The cancer-preventive effects of β-carotene combined with other chemicals (vitamin C, vitamin E, retinol, glutathione, oltipraz, 4-hydroxyphenylretinamide, selenium, wheat bran or perilla oil) were investigated in three studies in rats and one study in hamsters with regard to pancreatic carcinogenesis, in three studies on buccal pouch cancer in hamsters, in two studies on colon carcinogenesis in rats, in one study on respiratory tract tumours in hamsters and in one study on lung tumours in mice. In all of these studies, the combinations were as effective or more effective than β-carotene alone, except for one study in hamsters in which β-carotene alone or combined with vitamin C was ineffective in preventing pancreatic carcinogenesis and one study in mice in which β-carotene alone or in combination with retinol was ineffective in preventing lung tumours.

β-Carotene inhibited neoplastic transformation in four studies. The effect required continuous treatment during the post-initiation phase of carcinogenesis and was reversible after withdrawal of treatment. β-Carotene inhibited the formation of aberrant lesions in mouse mammary cells in one study. Maximum inhibition was seen when treatment was simultaneous and continued for the duration of the experiment.

Canthaxanthin

The cancer-preventive efficacy of canthaxanthin was assessed in several mouse, rat and hamster models. It prevented skin carcinogenesis in eight studies in mice in which ultraviolet B irradiation was used as the carcinogen; conflicting results were obtained in two studies in which 7,12-dimethylbenz[*a*]anthracene was the carcinogen. Canthaxanthin was effective in all six studies in hamsters in which the buccal pouch was the target organ. It showed cancer-preventive effects in one of four studies of liver

carcinogenesis in rats. The cancer-preventive efficacy of canthaxanthin was investigated in 10 studies in rats in models of cancers of the colon, mammary gland, tongue, small intestine, glandular stomach and salivary glands. In single studies, it was effective against cancers of the tongue and glandular stomach and ineffective against cancers of the salivary glands and small intestine. In studies of the colon and mammary gland, canthaxanthin was effective in one study and ineffective in another; the positive finding in mammary glands was associated with treatment before carcinogen administration. In two studies in mice, canthaxanthin had no preventive effect against urinary bladder cancer.

In one study in mice inoculated with malignant thymoma cells, canthaxanthin inhibited tumour growth when administered before and after tumour inoculation and appeared to be ineffective when given only after inoculation. It inhibited neoplastic transformation in cell cultures *in vitro*.

α-Carotene

In single studies in mice, α-carotene reduced the incidences of tumours of the liver, lung and skin, and in one study in rats it inhibited colon carcinogenesis. It inhibited neoplastic transformation *in vitro*.

Lycopene

A slight effect of lycopene was seen in two studies in rats in the aberrant crypt foci model of colon carcinogenesis. In models of liver carcinogenesis in rats, lycopene was ineffective in two studies and effective in one. In one study in mice, lycopene reduced the incidence of spontaneous mammary gland tumours but enhanced the development of preneoplastic mammary nodules. In another study, lycopene reduced the incidence of spontaneous liver tumours in mice. It was effective in one study of lung carcinogenesis in male but not female mice. It inhibited neoplastic transformation *in vitro*.

Lutein

In one study in rats, oral administration of lutein had preventive activity in the aberrant crypt foci model of colon carcinogenesis. In one study in mice, it was effective against skin carcinogenesis. In one study in mice inoculated with murine mammary tumour cells, lutein inhibited tumour growth. It inhibited neoplastic transformation *in vitro*.

Fucoxanthin

In single studies in mice, fucoxanthin applied to the skin was found to have preventive effects against skin tumours. Fucoxanthin in drinking-water inhibited the formation of tumours of the duodenum.

Mixtures

In four studies, *Spirulina–Dunaliella* extracts (containing 15–30% β-carotene, 20–25% xeaxanthin, 20–25% β-cryptoxanthin, 10–15% myxoxanthin, 10–15% echixenone and other carotenoids) were found to prevent cheek pouch carcinogenesis in hamsters. In one study in mice, diets supplemented with *Dunaliella* powder or *Dunaliella* extract (containing 0.03% β-carotene) reduced the incidence of spontanous mammary gland tumours. In single studies in mice, palm-oil carotene was effective in reducing the incidence of spontaneous liver cancer and of chemically induced carcinogenesis in the skin, lung, small intestine and stomach. In one study in rats, palm-oil carotene had no preventive effect in the aberrant crypt foci model of colon carcinogenesis.

8.3.2.2 Genetic and related effects

A number of carotenoids were evaluated for their ability to inhibit genetic and related effects *in vitro*. In most studies, carotenoids exerted protective effects against promutagens and mutagens that induce oxidative damage, whereas they did not affect the potency of directly acting mutagens. These findings were not always consistent, depending on the test compound and the laboratory that conducted the study. In addition to the usual limitations of in-vitro studies, resulting from the high concentrations of both genotoxic agents and modulators, the delivery system for carotenoids in these studies is very different from those *in vivo*.

In studies of the ability of orally administered carotenoids (mostly β-carotene) to

inhibit genetic and related effects in mice, rats and hamsters treated with a variety of physical or chemical carcinogens, the end-points included production of single-strand breaks in the DNA of liver or forestomach mucosa, mutations in T lympocytes, micronucleus formation or chromosomal aberrations in bone-marrow cells and binding to liver DNA. The results of most of the studies were consistent with protective effects of carotenoids.

8.3.3 Mechanisms of cancer prevention

The following mechanisms of action have been proposed or suspected to contribute to any cancer-preventive effects of carotenoids. Most of these mechanisms have been studied only *in vitro*, and more complex interactions among dietary components and mechanistic pathways are likely to occur *in vivo*.

All of the carotenoids examined inhibited oxidative or free-radical-initiated damage to synthetic or biological membranes. Processes involving free radicals and reactive oxygen species may be important at various stages of the multistep carcinogenic process. Carotenoids can interact with reactive oxygen species, and they have also been shown to inhibit lipid peroxidation.

In experimental models, carotenoids have been shown to prevent malignant transformation of normal cells or to induce cell differentiation. Carotenoids can stimulate gap-junctional communication between cells *in vitro*, an effect postulated to reduce the aberrant proliferation of carcinogen-initiated cells. In five studies, β-carotene at concentrations that were not cytotoxic was reported to decrease proliferation. In one study, lycopene was reported to be more effective than α- or β-carotene. In two reports, effects were seen in normal and dysplastic cells but not in cancer cells. The prevention of both malignant transformation and proliferation may be due to the formation of biologically active molecules from carotenoids. In rats, some carotenoids can modulate the activities of carcinogen-metabolizing enzymes.

Carotenoids have immunomodulating effects that could enhance cellular defence systems, possibly involving tumour-specific antigens. In three studies, β-carotene was reported to increase various parameters of immune responsiveness. In two studies, no increases were observed, although responses were reported in response to astaxanthin. Canthaxanthin was reported to increase immune responsiveness in one study but not in another in which different effector cells and end-points were used.

Both β-carotene and canthaxanthin increased expression of a receptor gene driven by the *RAR-β* promoter, but this finding was not confirmed in another study in which expression of the endogenous gene was studied. In human keratinocytes grown in organotypic culture, β-carotene and canthaxanthin decreased expression of mature *keratin 10* and increased expression of *connexin 43*.

Thus, carotenoids, because they may act in several different biological processes, should be considered nutritional modulators and not solely antioxidant or pro-oxidant molecules. Various mechanisms account for the observed protective effects, including delay in cell-cycle progression, decreased expression of proto-oncogenes, enhancement of intercellular communication, inhibition of metabolic activation of promutagens, enhancement of detoxification of reactive metabolites and inhibition of mutagenicity related to oxidative damage. The similar effects of carotenoids with and without provitamin A activity indicate a direct protective role *in vitro*.

8.4 Other beneficial effects

Antioxidants, including β-carotene and carotenoids, have been suggested to be of value in the prevention of a number of chronic diseases. The only current therapeutic use of carotenoids is in the treatment of erythropoietic protoporphyria, a photosensitivity disease. Although the results of a number of observational studies suggest that carotenoids may be of value in the prevention of cardiovascular disease, the results of the intervention trials with β-carotene do not support this hypothesis. Lutein and zeaxanthin have been suggested to play specific roles in the prevention of age-related macular

degeneration, but experimental data to support this hypothesis are not yet available. Senile cataract is another ocular condition potentially related to oxidation; carotenoids including β-carotene are being studied for a role in the prevention of this disorder, although the available results are somewhat inconsistent. Carotenoids have also been suggested to be of benefit for several other health outcomes including but not limited to ageing, impaired cognition, rheumatoid arthritis and cystic fibrosis; however, the data are scant.

8.5 Carcinogenic effects

8.5.1 Humans

The results of two intervention trials provide evidence suggesting that β-carotene increases the risk for lung cancer among current smokers. An increased risk for lung cancer was also seen in the trial that included workers who had been exposed to asbestos, most of whom were ex-smokers. Two other very large trials, which did not include large numbers of smokers or persons exposed to asbestos, did not show any increased risk for cancers at specific sites. The biological mechanism by which β-carotene at the doses used in the two trials may increase the risk for lung cancer in high-risk groups is not clear.

8.5.2 Experimental animals

β-Carotene was studied for carcinogenicity in one study in male and female rats and in one study in male and female mice, but the reports were available only in abridged form. Enhanced carcinogenesis was seen in isolated investigations of carotenoids administered in conjunction with carcinogens.

8.6 Other toxic effects

8.6.1 Humans

Hypercarotenodermia (discolouration of the skin) produced by intake of carotenoids at high doses is considered to be a reversible condition. Intake of high doses of supplemental β-carotene has been reported to increase the risk for death from cardiovascular disease in specific high-risk groups. Ingestion of canthaxanthin at high doses over long periods induces reversible retinopathy characterized by macular deposits.

There is no evidence to suggest that β-carotene is toxic at the levels found in most diets.

8.6.2 Experimental systems

In some studies of the induction of genetic and related effects *in vitro*, carotenoids were used as controls, generally at one dose only. None of the carotenoids tested, i.e. β-carotene, canthaxanthin, 8′-apo-β-carotenal, 8′-apo-β-carotenoyl methyl ester, carrot extract, carotenoids, cryptoxanthin and lycopene, was genotoxic in bacteria. Moreover, β-carotene did not induce sister chromatid exchange, chromosomal aberration or micronuclei in cultured mammalian cells.

β-Carotene has not been found to induce adverse genetic or related effects in experimental studies *in vivo*. It did not induce micronuclei in bone-marrow cells of mice, and it was neither clastogenic in rodent bone-marrow cells nor mutagenic in rat spleen T lymphocytes, although some conflicting data have also been reported. β-Carotene has been reported to enhance the hepatotoxicity induced by a high intake of alcohol in animals. It was not toxic to either embryos or dams in studies of developmental toxicity in rats and rabbits.