Pure & Appl. Chem., Vol. 63, No. 1, pp. 147–156, 1991.
Printed in Great Britain.

206922209100016

Recent progress in the medical applications of carotenoids

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Abstract - Carotenoids continue to be important dietary sources of vitamin A, and also continue to be used in the treatment of certain human photosensitive skin diseases. Animal and epidemiologic studies are strongly suggestive that carotenoids may have an anti-cancer role, but the clinical prevention trials designed to determine if this is true have not yet been completed. Another interesting possible role of carotenoids is as modulators of immunologic responses: investigations on this aspect of carotenoid function are also ongoing. In this paper we survey the recent literature on these diverse roles of carotenoids.

INTRODUCTION

At the last three International Symposia on Carotenoids, there have been discussions of the medical uses, other than in nutrition, of these pigments. Although the acknowledged primary use of carotenoids is in nutrition, it is now well established that the pigments act as photoprotective agents, and this property is made use of in the treatment of certain skin diseases. In addition, there is some evidence, though not as firm as that for their photoprotective role, that carotenoids may have a role in preventing certain forms of cancer. The chemical mechanisms whereby the pigments perform these various roles are also of interest. We continue to discover new effects that these fascinating compounds have in biological systems.

PHOTOPROTECTION

Twenty years ago, we first reported that the administration of large doses of β-carotene to patients suffering from the genetic disease of porphyrin metabolism, erythropoietic protoporphyria (EPP), could prevent or ameliorate the photosensitivity associated with this condition (refs. 1 & 2). The use of β-carotene for the treatment of photosensitivity in EPP was approved by the United States Food and Drug Administration in 1975. Since that time, many authors have reported on this use of carotenoids, which has recently been reviewed (refs. 3 & 4). Reports continue to appear in the literature of the successful use of β-carotene in EPP, as well as in other photosensitivity diseases (refs. 5-8).

In an animal model, Kornhauser et al. (ref. 9) have found that β-carotene administration can significantly prevent the development of skin reddening (erythema) induced by ingestion of 8-methoxypsoralen followed by UV-A radiation (320-400 nm: PUVA). Unfortunately, β-carotene does not seem to prevent PUVA erythema in man, or erythema caused by UV-A alone, or by UV-B (290-320 nm) (refs. 10-12).

β-Carotene also prevents the photosensitivity associated with quinidine ingestion (ref.13). It also prevented the depletion of glutathione in the brainstem by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice, but not in marmosets (refs. 14 & 15), perhaps indicating a species difference of penetration of carotenoids and α-tocopherol into the brain.

Roe (ref. 16) has reported that blood carotenoid levels decrease after multiple exposures of UV-A and UV-B light. Further studies are needed to determine if exposure to natural sunlight has a similar effect on serum carotenoid levels, and if this would happen with carotenoid supplementation.

Prince et al. (refs 17 & 18) have taken advantage of the fact (ref. 19) that atherosclerotic plaques accumulate carotenoids, whereas the adjoining normal artery wall does not, to suggest that β-carotene could be administered to patients undergoing laser coronary angioplasty to make this treatment more efficient, by increasing the contrast between plaque and normal artery wall.

Among the antioxidant functions of carotenoids (ref. 20) is their ability to act as antioxidants in conditions other than photosensitization. Recent examples of such studies are that of Mayne and Parker (ref. 21) which showed that dietary canthaxanthin increased resistance to lipid peroxidation, and that of Blakeley et al. (ref. 22), which found that rats fed β-carotene had lower levels of high fat diet-induced superoxide dismutase and catalase activity in the liver. Antioxidant activity of carotenoids in the absence of light will be an area of increasing research activity in the years to come.

CAROTENOIDS AND CANCER PREVENTION

Epidemiologic studies

Peto et al. (ref. 23), though they did not originate the idea, convincingly summarized the available evidence that β-carotene might be effective in preventing cancer development. Their publication provided the momentum to get additional epidemiological and experimental studies funded and performed. The more recent epidemiologic data seem to be the most firm for lung cancer (refs. 24-29). There may also be a protective effect of carotenoids against certain other cancers, such as laryngeal cancer (ref. 30), gastric cancer (ref. 31), cervical dysplasia and cervical cancer (ref. 32), and invasive bladder cancer (ref. 33), but the association is not as definite as for lung cancer. In contrast, no risk reduction by either carotenoids or retinol was found for the development of esophageal cancer (ref. 34), breast cancer (ref. 35), or head, neck and gastrointestinal cancer (ref. 36). In most cases, a protective function for retinol has not been found.

There is continued concern about the effects of smoking on serum carotenoid levels. Gerster (ref. 37) showed that smoking and Aoki et al. (ref. 38) that smoking and drinking can decrease blood carotenoid levels. Sidney et al. (ref 39) found that dietary intake of carotenoids was lower in nonsmokers exposed to passive smoke than in nonsmokers not exposed to passive smoke, and stressed that it is important to determine carotenoid intake as well as serum levels in all groups involved in carotenoid cancer prevention studies.

Many studies have shown that women who smoke during pregnancy have a high probability of having a low birth-weight infant. Metcoff et al. (ref. 40) found that in women in the second trimester of pregnancy who were smokers, the relationship of cholesterol to birth weight depended on the carotenoid level: if carotenoid and cholesterol levels were low, the birth weight was low, and if the carotenoid level was high and the cholesterol low, the birth weight was equal to that in non-smokers. Smokers whose carotenoid levels stayed the same or rose had the largest babies. The authors feel that carotenoids and cholesterol have a positive effect on fetal growth in non-smokers, and that carotenoids have a positive effect in smokers.

Human intervention studies

At present, there are twelve human intervention studies in progress to see if the administration of β-carotene can prevent the development of various kinds of cancer, but none of these large-scale studies is completed at this time. In smaller-scale studies, Stich et al. (refs. 41 & 42) reported that in individuals who ingested β-carotene for 6 months, there was a significant reduction in the number of micronucleated buccal cells, as well as a significant remission and inhibition of new oral leucoplakias. Also, Guillot et al. (ref. 43) treated a 7-year-old child suffering from xeroderma pigmentosum with a retinoid, with some improvement: when the combination of β-carotene and canthaxanthin (Phenoro, Roche) was added to the regimen, no new lesions had developed after a year of the combined therapy. In addition, β-carotene administration prevented the oral mucinosis which developed after radiation and chemotherapy in patients with oral cancers (ref. 44).

Cell culture studies

The protective effect of carotenoids, irrespective of the pigments' vitamin A activity, can be demonstrated in cell culture systems. Recent as well as past studies have produced additional evidence that the pigments can prevent

malignant transformation (refs. 45 & 46), sister chromatid exchange and other chromosomal changes in cell cultures, as well as mutagenic effects in bacterial systems (ref. 47). On the other hand, Garewal et al. (ref. 48) found that neither β-carotene, canthaxanthin, nor retinal had any effect in inhibiting the growth of cells in culture from lesions of Barret's esophagus, a pre-malignant condition of the esophagus, with an increased risk of esophagal carcinoma. They also reported that retinoic acid had no effect in a small clinical trial in patients with this condition.

The ability of carotenoids to prevent or delay the development of UV-B-induced or chemically-induced skin tumors in animal models is now well established (refs. 1, 2, & 20). In other animal cancer models, certain carotenoids inhibited development of gastric mucosal lesions produced by HCl application (ref. 49), purified β-carotene and an algal extract (Phycotene) rich in carotenoids caused regression of an established fibrosarcoma in the mouse (ref. 50), a combination of β-carotene and α-tocopherol given orally caused regression of established epidermoid carcinomas in the hamster buccal pouch model (ref. 51), β-carotene supplementation decreased the development of preneoplastic and neoplastic lesions induced by N-nitrosobis(2-oxopropyl) amine and azaserine in exocrine pancreas of rats and hamsters (ref. 52), and crocin dyes afforded some protection against acute hepatic damage in rats caused by aflatoxin B and dimethylnitrosamine (ref. 53). On the other hand, β-carotene offered no protection against the development of stomach or small intestinal tumors in mice, or colo-rectal cancer in rats (refs. 54 & 55), but one study (ref. 56) does report protection against colon tumors in mice.

Nagasawa et al. (ref. 57) recently reported that mice given a concentrated algal preparation rich in β-carotene developed fewer spontaneous mammary tumors. Grubbs (personal communication) has found that canthaxanthin could significantly prevent 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary cancers in rats if the pigment were fed before DMBA administration, but not if canthaxanthin feeding was started 2 weeks after the administration of the carcinogen.

The author also studied the ability of β-carotene and canthaxanthin to prevent the development of N-methyl-N-nitrosourea (MNU)-induced breast tumors in rats. At 25 days of age, the animals were placed on an unpurified diet (Purina Chow) containing either 1% canthaxanthin (canthaxanthin beadlets, Roche), 1% β-carotene (β-carotene beadlets, Roche) or placebo beadlets. At 50 days of age, the animals received 50 mg/kg of MNU (Sigma Chemical Co.) dissolved in buffered saline, according to the technique of Thompson and Meeker (ref. 58). After an observation period of 250 days, no tumors had developed, so the animals received an additional dose of 50 mg/kg of MNU (Ash-Stevens Chemical Company). Tumors developed, and the animals were sacrificed at 568 days of age. It was found that 5 of the 19 rats in the placebo group had developed carcinomas of the breast, but only one of the 19 mice in the canthaxanthin-treated group had developed a carcinoma. This suggested a trend, but was not statistically significant. Four out of 20 rats receiving β-carotene developed tumors, suggesting that no protection was offered by this pigment. The number of tumors developed in the placebo and β-carotene groups is higher than the rate of spontaneous tumor development in the rat, and would indicate that the second dose of carcinogen was effective, and in addition shows that cancers can be induced in older animals, though at a rate somewhat lower than in younger ones (H.J. Thompson, personal communication). These results confirm those of Grubbs et al. and Nagasawa et al., but additional studies are warranted to determine what effects, if any, carotenoids have in the prevention of breast cancers.

Several studies have investigated other properties of carotenoids. Edes et al. (ref. 59) found that β-carotene caused a higher activity of intestinal, but not liver, aryl hydrocarbon hydroxylase in rats fed β-carotene, than in rats fed vitamin A. Mikhailenko et al. (ref. 60) found that β-carotene given to rats before they received the carcinogen N-nitrosodimethylamine not only decreased the methylation and formation of single-strand breaks in DNA, but also decreased activity of alanine-aminotransferase, sorbitol dehydrogenase, and gamma-glutamyl transpeptidase. Holloway and Gainer (ref. 61) reported that the administration of crocetin enhances pulmonary oxygenation in anesthetized rabbits, and cerebral oxygenation in hemorrhaged rats.

DeLuca et al. (ref. 62) stress that, in the design of cancer experiments, it is crucial to provide all animals with physiological amounts of retinoids, or their carotenoid precursors: if this is not done, tumors will not develop, and false results of a compound's cancer-preventive ability may be obtained.

How carotenoids might prevent cancer is also being investigated. Wolf et al. (ref. 11) studied unscheduled DNA synthesis in human subjects exposed to UV-B light, and found no difference in unscheduled DNA synthesis between those who received carotenoids and those who received placebo. They concluded that the cancer-preventive effects of carotenoids do not occur by preventing the induction of DNA dimers or other lesions that are reversible by excision repair.

CAROTENOIDS AND THE IMMUNE SYSTEM

Bendich, at the last Carotenoid Symposium, and in a recent publication, (refs. 63 & 64), has reviewed the role of carotenoids in modulating immunological reactions. The pigments have been found by several workers to enhance both specific and non-specific immune functions, as well as to enhance tumor immunity. It is important to note that pigments with and without vitamin A activity are effective. She postulates that carotenoids may enhance immune activity by: 1) quenching excessive reactive species formed by various immunoactive cells, 2) quenching immunosuppressive peroxides and maintaining membrane fluidity, 3) helping to maintain membrane receptors essential for immune function and 4) acting in the release of immunomodulatory lipid molecules such as prostaglandins and leukotrienes. These various mechanisms can increase the tumoricidal activity of cytotoxic T-cells, macrophages, and/or natural killer cells, as well as enhance traditional antimicrobial immunological function.

Work continues on this most interesting aspect of carotenoid function. Prabhala et al. (ref. 65), in a study of the effects of retinoids, β-carotene and canthaxanthin on circulating T-lymphocytes and natural killer cells, found that carotenoids and retinoids, which both have effects on cells of the immune system, may be activating different subpopulations of immune cells. Abril et al. (ref. 66) showed that β-carotene stimulated peripheral blood leucocytes to secrete a novel cytokine, which caused the lysis of six human tumor cell lines, but was not toxic to normal fibroblasts. Schoen and Watson (ref. 67) have demonstrated that β-carotene can protect isolated monocytes from UV-B induced damage to phagocytic activity. Gensler (ref. 68) recently found that dietary supplementation with retinyl palmitate plus canthaxanthin prevented the transfer of UV-induced immunosuppression with splenocytes from UV-irradiated mice.

CAROTENOIDS AND NUTRITION

Metabolism

The question of the importance of eccentric versus central cleavage of the carotenoid molecule to form retinal is not yet settled (refs. 69 & 70): studies of carotenoid metabolism continue. Hansen and Maret (ref. 71) could not demonstrate central cleavage using rat intestine extracts (though others continue to do so) but did find small amounts of β-apocarotenals formed nonenzymatically. Villard and Bates (ref. 72) suggest that low tissue vitamin A levels may feedback to increase carotenoid dioxygenase activity. Napoli and Race (ref. 73) examined intestine, liver, kidney, lung and testes for their ability to form retinoic acid and retinal from β-carotene, and found that intestine formed less product than the other tissues. The formation was definitely an enzymatic reaction, as boiling the tissue extract resulted in no products being formed. The authors conclude that the tissues' ability to form retinoic acid from β-carotene contributes significantly to their needed supply of retinoic acid, and that this "in situ" formation may be important in those anti-cancer functions attributed to vitamin A activity, as opposed to functions attributed to the intact carotenoid molecule.

Pharmacokinetic studies

Dimitrov et al. (ref. 74) stress that it is important to realize that the bioavailability of carotenoids is associated with much inter- and intra-individual variations, and that these differences can be very important when small pharmacological doses of carotenoids are given in large intervention trials. Here, a combination of low absorption and poor compliance, as well as long intervals between measurements, may lead to misinterpretation of the results. He suggests that larger intermittent doses, rather than small daily doses, may lead to the maintenance of significant plasma levels without discoloring skin, and that this would eliminate compliance problems. The physical condition of the individual trial participants, such as large body

surface area, or a large fat mass, should also be considered in establishing the carotenoid dose for intervention trials. In addition, he pointed out that micronutrients pose a special problem for the determination of half-time clearance, largely because of interference from micronutrients present in food. The half-time clearance for 15 and 30 mg β-carotene could not be calculated, but that for 45 mg was found to be 4-5 days and that for 150 mg was 5-6 days.

Several other workers have followed the appearence of carotenoids in blood using various dosage schedules of either β-carotene or canthaxanthin. Meyer et al. (ref. 75) administered either 4 or 6 capsules of β-carotene/ canthaxanthin (Phenoro) to normal volunteers for 17 days and found that blood carotenoid levels in both groups reached a plateau at essentially the same time, though the group receiving the 6 capsules had a higher absolute level of both carotenoids, and that the subjects could be divided into low and high absorbers. Costantino et al. (ref. 76) administered 15 mg/day of β-carotene for one year to 300 men enrolled in a pilot study for a lung cancer intervention trial, and found that this dose produced a ten-fold increase in serum β-carotene levels with no toxicity or significant skin discoloration.

Brown et al. (ref. 77) studied the plasma levels of 7 carotenoids in 30 men who had taken a single dose of either 12 mg β-carotene, 30 mg β-carotene, 270 g carrots, 600 g broccoli or 180 g tomato juice, and found that only the β-carotene and carrots resulted in a detectable increase in plasma β-carotene, which occurred 24-48 hours after intake: vegetable ingestion did not change the levels of any of the carotenoids studied. They also found great individual variation between subjects, and also concluded that plasma response to pure β-carotene is greater than the response to a similar amount of β-carotene in carrots, confirming previous findings of other workers. Micozzi et al. (ref. 78) gave 30 volunteers either 12 mg β-carotene, 30 mg β-carotene, 272 g carrots, 300 g broccoli, 180 g tomato juice or placebo for 42 days. The investigators found definite carotenodermia by clinical observation of the skin only in the 5 subjects who took the 30 mg of pure β-carotene, although plasma analysis revealed some increase in β-carotene in all groups, and an increase in lycopene in the group receiving tomato juice. Carotenodermia first appeared between 25 and 42 days after supplementation started, persisted for 14 to more than 42 days after supplementation stopped, and was seen only after plasma β-carotene levels were greater than 400 μg/dl. Questionable carotenodermia was seen on at least one examination in 4 of the 5 men who took the 12 mg β-carotene capsules, in 3 of the 5 men in the carrot group, in two of the 5 men in the broccoli group, and in one of the 5 men in the placebo group. No subject in the group receiving tomato juice developed carotenodermia, although coloration from the principal pigment in tomatoes, lycopene, has been described (ref. 79). A questionnaire given to the subjects revealed that 4 of the 5 men in the 30 mg β-carotene group and one each from the groups consuming carrots and broccoli described themselves as having a change in their skin color: 3 had noticed it themselves, and the other 3 had it pointed out by another person. They did not seem to dislike the color. This confirms our previous experience with volunteers in a β-carotene and tanning study (ref. 12), many of whom communicated that they wanted to continue carotenoid intake because they liked the color it gave their skin. It has also been our experience that most patients with EPP do not mind the carotenodermia.

Jensen et al. (ref. 80) administered to human volunteers either 1) capsules containing β-carotene isolated from Dunaliella salina and containing a total of 9.6 mg of trans-β-carotene and 14.4 mg of cis-β-carotene, suspended in vegetable oil, 2) an amount of carrots containing 23.5 mg of trans-β-carotene and 0.5 mg of cis-β-carotene, or 3) placebo capsules. The authors found a predominant absorption of intact trans-β-carotene over intact cis-β-carotene into serum, even when approximately equivalent amounts of isomers were ingested, and concluded that the intact trans form is preferentially absorbed over intact cis-β-carotene in the human subject, in spite of the fact that there was some increased absorption of the cis isomer in the groups receiving supplementation. They suggest several possibilities to explain their findings: 1) most of the cis-β-carotene is unabsorbed, 2) cis-β-carotene is preferentially converted to vitamin A in the intestinal mucosa, or 3) some isomerization of cis- to trans-β-carotene occurs.

Nierenberg and Stuckel (ref. 81) studied the diurnal variation of serum levels of β-carotene, retinol and α-tocopherol in adult volunteers following

their usual diets and not taking any carotenoid or vitamin supplements. They found that retinol levels decreased between 11 AM and 5 PM, but β-carotene and α-tocopherol levels showed no change in pattern during the day.

Carotenoid accumulation in organs

Although there are no detailed studies of carotenoid accumulation in all human tissues, from the data available it seems that the principal stores are adipose tissue and liver, with smaller amounts in other tissues, such as skin (ref. 69). The major carotenoids in human serum are β-carotene, α-carotene, cryptoxanthin, lycopene, and lutein. Small amounts of zeaxanthin, phytofluene and phytoene may also be found in various organs. Parker (ref. 82) studied the carotenoid composition of human adipose tissue, and found that β-carotene and lycopene were the predominant pigments, lycopene levels sometimes exceeding those of β-carotene, with much individual variation.

Carotenoids were known to exist in the retinas of primates, but definite identification was not done until the work of Bone et al. (refs. 83 & 84), and Handelman et al. (ref. 85), who have studied retinal pigments in eyes from subjects of various ages, and have found both lutein and zeaxanthin in the retinas of fetuses, children and adults. A visible macular spot was not seen until about 6 months after birth. The quantity of pigment does not seem to vary much with age, though there is individual variation. Zeaxanthin was found to be the dominant pigment in most of the maculas. There seems to be a difference in the location of each pigment over the surface of the retina, with zeaxanthin more centrally and lutein more peripherally located, suggesting that cone cells might accumulate zeaxanthin and rod cells, which are located more in the periphery of the retina, might accumulate lutein.

Other studies

Singh et al. (ref. 86) reported an increase in plasma total carotenoid levels in elderly females with Alzheimer's disease. The patients had a mean of 140 μg/dl, with a range of 55 to 275 μg/dl. Non-demented elderly women of the same age and at the same institution had a mean level of 78μg/dl, with a range of 35 to 150 μg/dl. Women also hospitalized there with multi-infarct dementia had a mean plasma level of 91 μg/dl, with a range of 30 to 300 μg/dl. However, no details on the diets of the three groups were given.

Clemens et al. (ref. 87) found that patients being maintained on total parenteral nutrition in preparation for bone marrow transplantation had low serum levels of β-carotene, confirming previous findings that parenteral diets are low in carotenoids. They suggest that low carotenoid levels might be harmful in that high anti-oxidant levels might prevent some of the unwanted side effects of radiation and chemotherapy treatment.

Anzo and Moore (ref. 88) used photoacoustic techniques to determine in which layer of human skin β-carotene is deposited. They found that most was deposited in the stratum corneum, which confirmed findings from chemical extraction of skin (refs. 89 & 90).

Ribaya-Mercado et al. (ref. 91) have suggested that the ferret is a more appropriate animal model of carotenoid absorption than is the rat, because these animals absorb and store higher levels of β-carotene than do rats fed the same amounts of pigments.

In another animal study, Alam et al. (ref. 92) found that plasma β-carotene levels were significantly reduced in rats fed corn oil as compared to those fed the same amount of lard, suggesting that the kind of dietary fat ingested can significantly affect the circulating and tissue levels of β-carotene. Plasma vitamin A levels were unaffected, but liver vitamin A levels were significantly elevated in both fat-fed groups, being higher in lard-fed rats.

Cutler (ref. 93) suggests that, since β-carotene concentrations in serum and brain tissue show a positive correlation with the maximum life-span potential of mammalian species, carotenoids may be biologically active not only as a protective agent against cancer, but also as a longevity determinant. Retinol did not show this correlation. However, Massie et al (ref. 94) did not find any effect on lifespan prolongation of 0.5% β-carotene supplementing standard laboratory feed for mice, the supplementation starting at 29 days of age and lasting for the rest of the animals' lives.

TECHNICAL ASPECTS OF CAROTENOID DETERMINATION

Two recent studies investigated the effects of handling and storage of specimens on the determination of serum or plasma carotenoid levels. Craft et al. (ref. 95) found no significant difference in the concentrations of a variety of carotenoids (lutein/zeaxanthin, cryptoxanthin, lycopene, β-carotene and α-carotene), retinol and α-tocopherol between plasma frozen immediately and duplicate samples kept at room temperature in the dark for 24 hours, and that the compounds under study were stable in solvents for at least 18 hours at 23° C after extraction, confirming similar observations by the author and Stampfer (ref. 96). Purging samples with nitrogen before freezing had no noticeable beneficial effect. They found that the carotenoids studied were stable in plasma stored at -70° C for at least 28 months, or at -20° C for 5 months, but by 15 months the concentration was significantly less in plasma stored at -20° C than at -70° C. Adding antioxidants (ascorbic acid, butylated hydroxytoluene) did not prevent the deterioration. However, retinol and α-tocopherol did not undergo this deterioration. Thurnham and Flora (ref. 97) have confirmed the findings of Craft et al., showing that β-carotene, lycopene and β-cryptoxanthin decreased significantly on storage at -20° C, but α-carotene did not. These two studies lead to the conclusion that any serum or plasma samples stored at -20° C must be analyzed within 5 months for the data to be considered reliable.

Gunson et al. (ref. 98) warned that plasma or serum specimens from individuals ingesting carotenoids, especially canthaxanthin, may be bright orange, confirming the findings of Bareford et al. (ref. 99). In such specimens, tests for hemolysis or anemia or bilirubin will be within the normal range and extraction of the specimen with ethanol and petroleum ether will reveal the presence of high levels of carotenoid(s). Questioning the blood donors will usually reveal the ingestion of large amounts of canthaxanthin pills, or, more rarely, β-carotene pills or large amounts of vegetables.

It was pointed out by Thompson (ref. 100) that high plasma and serum carotenoid levels can interfere with the fluorometric analysis of vitamin A. This can also happen in some of the spectrophotometric analyses for this vitamin. In both kinds of tests, it is imperative that correction factors for blood carotene levels be applied, especially in the analysis of specimens from large intervention studies where carotenoids are administered, so that erroneous reports of high vitamin A levels will not be made. It is well established that humans do not make abnormal amounts of vitamin A from ingested β-carotene.

LACK OF TOXICITY OF CAROTENOIDS

The primary "toxicity" reaction from the intake of carotenoids remains the discoloration of the skin (carotenodermia): as we have mentioned above, most individuals taking supplementary carotenoids do not mind the skin color, and some actually like it. There is no evidence that carotenoids, as represented by β-carotene, are mutagenic or have embryotoxicity (refs. 101 & 102). Also, β-carotene supplementation does not elevate triglyceride levels (ref. 103) or cholesterol levels (ref. 104).

Another area of concern has been the crystalline deposits formed in the retinas of individuals taking large cumulative doses of canthaxanthin. In some people, the deposits were associated with decreased dark adaptation. No significant decrease of visual acuity has been reported. The evidence now available (refs. 105 - 108) indicates that these deposits will eventually disappear with cessation of canthaxanthin intake, though in some individuals this may take several years. In those who had suffered changes in dark adaptation, vision returned to normal soon after cessation of canthaxanthin intake, and before the crystals disappeared.

Lumikari et al. (ref. 109) examined the effects of β-carotene ingestion on the secretion of salivary glycoproteins and some antibacterial components of saliva, and found that subjects receiving the β-carotene supplementation had a significantly higher level of pigment in whole saliva than did those receiving placebo. Saliva retinol levels were unchanged. Saliva collected directly from the parotid gland duct did not contain either β-carotene or retinol. Whole saliva β-carotene levels correlated with serum levels: no such correlation existed for retinol. They also found that β-carotene

supplementation did not affect the secretion rate of whole saliva, nor the content of total protein, specific proteins or glycoconjugated carbohydrates, lysozyme, secretory IgA, or bacteria-aggregating glycoprotein in stimulated whole saliva or parotid saliva.

CONCLUSIONS

The photoprotective and antioxidant properties and functions of the intact carotenoid molecule are well established, although the actual mechanisms of action still need to be completely described. It is too early to conclude that carotenoids can play a significant role in cancer prevention: we must await the results of the ongoing clinical intervention trials. Also, the significance of the pigments' immunomodulatory effects on intact animals still needs to be investigated, and mechanisms of action established. Even the metabolism of carotenoids is not completely understood, and more work needs to be done on this question also. Thus, we are left with much exciting and important research to do on the medical aspects of carotenoids, which hopefully will result in many more interesting sessions at future International Carotenoid Symposia.

REFERENCES

1. M.M. Mathews-Roth, M.A. Pathak, T.B. Fitzpatrick, L.C. Harber and E.H. Kass, New Engl. J. Med. 282, 1231-1234 (1970).
2. M.M. Mathews-Roth, M.A. Pathak, T.B. Fitzpatrick, L.C. Harber and E.H. Kass, Trans. Assoc. Am. Physicians 83, 176-184 (1970).
3. M.M. Mathews-Roth, in E. Ben-Hur and I. Rosenthal, eds, Photomedicine pp. 63-85, CRC Press, Boca Raton (1989).
4. M.M. Mathews-Roth, in G.A. Spiller and J. Scala, eds, New Protective Roles for Selective Nutrients, pp. 17-38, A.R. Liss, New York, (1989).
5. J. Barth, E. Fickweiler, K. Harnack, K. Herrmann, U. Hubner, H. Schaarschmidt and F. Schiller, Dermatol. Monatschr. 170, 244-248 (1984).
6. C.I. Harrington, Brit. J. Dermatol. 115, Suppl. 30, 87-88 (1986).
7. C.T. Jansen, Photodermatol. 2, 166-169 (1985).
8. W.P. Rabb, H. Tronnier and A. Wiskemann, Dermatologica 171, 371-373 (1985).
9. A. Kornhauser, W. Wamer and L. Lambert, in N.I. Krinsky, M.M. Mathews-Roth and R.F. Taylor, eds, Carotenoids: Chemistry and Biology, pp. 301-312, Plenum Press, New York (1990).
10. K. Macdonald, G. Holti and J. Marks, Dermatologica 169, 41-46 (1984).
11. C.Wolf, A. Steiner and H. Honigsmann, J. Invest. Dermatol. 90, 55-57 (1988).
12. M.M. Mathews-Roth, M.A. Pathak, J. Parrish, T.B. Fitzpatrick, E.H. Kass, K. Toda and W. Clemens, J. Invest. Dermatol. 59, 349-353 (1972).
13. D.A. Fisher, Arch. Dermatol. 120, 298, (1984).
14. V.W. Yong, T.L.Perry and A.A. Krisman, Neurosci. Lett. 63, 56-60 (1986).
15. T.L. Perry, V.W. Yong, S. Hansen, K. Jones, C. Bergeron, J.G. Foulks and J.M. Wright, J. Neurol. Sci. 81, 321-331 (1987).
16. D.A. Roe, Federation Proc. 46, 1886-1889 (1987).
17. M.R. Prince, T.F. Deutch, M.M. Mathews-Roth, R. Margolis, J.A. Parrish, and A.R. Oseroff, J. Clin. Invest. 78, 295-302 (1986).
18. G.M. LaMuraglia, M.M. Mathews-Roth, J.A. Parrish, W.M. Abbot, D.C. Brewster, D.J. McAuliffe and M.R. Prince, J. Vasc. Surg. 9, 563-567 (1989).
19. D.H. Blankenhorn, Ann. Int. Med. 53, 944-954 (1960).
20. N.I. Krinsky, Free Radical Biol. Med. 7, 617-635 (1989).
21. S.T. Mayne and R.S. Parker, J. Nutr. Cancer 12, 225-236 (1989).
22. S.R. Blakeley, L. Slaughter, J. Adkins and E.V. Knight, J. Nutr. 118, 152-158 (1988).
23. R. Peto, R. Doll, J.E. Buckley and M.B. Sporn, Nature 290, 201-208 (1981).
24. R.G. Ziegler, J. Nutr. 119, 116-122 (1989).
25. G.A. Colditz, M.J. Stampfer and W.C. Willett, Arch. Int. Med. 147, 157-160 (1987).
26. N.J. Wald, S.G. Thompson, J.W. Densen, J. Boreham and A. Bailey, Brit. J. Cancer 57, 428-433 (1988).
27. H.B. Stahelin, F. Gey and G. Brubacher, Int. J. Vit. Nutr Res. Suppl. 30, 232-241, (1989).
28. G.A. Kune, S. Kune, L.F. Watson, R. Pierce, B. Field, L. Vitella, D. Merenstein, A. Hayes and L. Irving, Nutr. Cancer, 12, 169-176 (1989).

29. J.E. Connett, L.H. Kuller, M.O. Kjelsberg, B.F. Polk, G. Collins, A. Rider, S.B. Hulley, Cancer 64, 126-134 (1989).
30. D. Mackerras, P.A. Buffler, Am. J. Epidemiol. 123, 980-988 (1988).
31. S. Graham, B. Haughey, J. Marshall, J. Brusure, M. Zielezny, J. Freudenheim, P. West, J. Nolan and G. Wilkinson, Nutr. Cancer 13, 19-34 (1990).
32. P.R. Palan, M. Mikhail and S.L. Romney, Am. J. Obstet. Gynecol. 161, 1649-1652 (1989).
33. H.A. Tyler, R.G. Notley, F.A.W. Schweitzer and J.W.T. Dickerson, Europ. J. Surg. Oncol. 12, 35-41 (1986).
34. W. Guo, J-Y. Li, W.J. Blat, A.W. Hsing, J. Chen and J.F. Fraumenti, Nutr. Cancer 13, 121-127 (1990).
35. E. Marubini, A. DeCarli, A. Costa, C. Mazzoleni, C. Andreoli, A. Barbieri, E. Capitelli, M. Carlucci, F. Cavullo, N. Monferrari and S. Salvini, Cancer, 61, 173-180 (1988).
36. M.M. Meguid, A.M. Landel, L. Oey and D.S. McLaren, J. Parenteral Enteral Nutr. 12, 147-151 (1988).
37. H. Gerster, J. Nutr. Growth Cancer 4, 45-49 (1987).
38. K. Aoki, Y. Ito, R. Sasaki, M. Otani, N. Hamajina, and A. Asano, Jap. J. Cancer Res. (Gann.) 78, 1049-1056 (1987).
39. S. Sidney, B.J. Caan, G.D. Friedman, Am. J. Epidemiol. 129, 1305-1309 (1989).
40. J. Metcoff, P. Castiloe, W.M. Crosby, H.H. Sandstead and D. Milne, Obstet. Gynecol. 74, 302-309 (1989).
41. H.F. Stich, M.P. Rosin, A.P. Hornby, B. Mathew, R. Sankaranarayanan and M. Krishnan Nair, in N.I. Krinsky, M.M. Mathews-Roth and R.F. Taylor, eds, Carotenoids: Chemistry and Biology, pp. 313-322, Plenum Press, New York, (1990).
42. H.F. Stich, M.P. Rosin, A.P. Hornby, B. Mathew, R. Sankaranarayanan and M. Krishnan Nair, Int. J. Cancer, 42, 195-199 (1988).
43. B. Guillot, C. Favier, J.J. Guilhou and J. Meynadier, Ann. Dermatol. Venereol. 111, 65-67, (1984).
44. E.E.D. Mills, Brit. J. Cancer 57, 416-417 (1988).
45. K. Manoharan, S. Som, M. Chatterjee and M.R. Banerjee, in N.I. Krinsky, M.M. Mathews-Roth and R.F. Taylor, eds, Carotenoids: Chemistry and Biology, pp. 293-300, Plenum Press, New York, (1990).
46. A. Pung, J.E. Rundhaug, C.N. Yoshizawa and J.S. Bertram, Carcinogenesis, 9, 1533-1539 (1988).
47. T. Ong, W-Z Whong, J.D. Stewart and H.E. Brockman, Mutat. Res. 222, 19-25 (1989).
48. H. Garewal, R. Prabhala, D. Sloan and R. Sampliner, Preventive Med. 17, 244 (1988).
49. T. Javor, M. Bata and L. Lovasz, Int. J. Tissue Rea. 5, 289-296 (1983).
50. W. Combs, S.T. Sonis, J. Fitzgerald, C. Tracy and R. Wilson, Nutr. Cancer 12, 371-380 (1989).
51. G. Shklar, J. Schwartz, D. Trickler and S. Reid, Nutr. Cancer 12, 321-325 (1989).
52. R.A. Wouterson and A. vanGarderen-Hoetmer, Cancer Lett. 42, 79-85 (1988).
53. J.K. Lin and C.J. Wang, Carcinogenesis 7, 595-599 (1986).
54. R.C. Jones, S. Sugie, J. Braley and J.H. Weisburger, J. Nutr. 119, 508-514 (1989).
55. T.A. Colacchio, V.A. Memoli and L. Hildebrandt, Arch. Surg. 124, 217-221 (1989).
56. N.J. Temple and T.K. Basu, J. Nat. Cancer Inst. 78, 1211-1214 (1987).
57. H. Nagasawa, R. Konishi, N. Sensui, K. Yamamoto and A. Ben-Amotz, Anticancer Res. 9, 71-76 (1989).
58. H.J. Thompson and L.D. Meeker, Cancer Res. 43, 1628-1629 (1983).
59. T. Edes, W. Thornton and J. Shah, J. Nutr.. 119, 796-799 (1989).
60. V.M. Mikhailenko, M.M. Vilenchik and M.A. Furman, Byull. Eksp. Biol. Med. 106, 481-483 (1988).
61. G.M. Holloway and J.L. Gainer, J. Appl. Physiol. 65, 683-686 (1988).
62. L.M. DeLuca, R.L. Shores, E.F. Spangler and M.L. Wenk, Cancer Res. 49, 5400-5406 (1989).
63. A. Bendich, in N.I. Krinsky, M.M. Mathews-Roth and R.F. Taylor, eds, Carotenoids: Chemistry and Biology, pp. 323-336, Plenum Press, New York, (1990).
64. A. Bendich, J. Nutr. 119, 112-115 (1989).
65. R.H. Prabhala, V. Maxey, M.J. Hicks and R.R. Watson, J. Leukoc. Biol. 45, 249-254 (1989).
66. E.R. Abril, J.A. Rybski, P. Scuderi and R.R. Watson, J. Leukoc. Biol. 45, 255-261 (1989).
67. D.R. Schoen and R.R. Watson, Photochem. Photobiol. 48, 659-663 (1988).
68. H.L. Gensler, Carcinogenesis 10, 203-207 (1989).

69. J.A. Olson, J. Nutr. 119, 105-108 (1989).
70. J. Ganguly and P.A. Sastry, World Rev. Nutr. Diet. 45, 198-220 (1985).
71. S. Hansen and W. Maret, Biochemistry 27, 200-206 (1988).
72. L. Villard and C.J. Bates, Brit. J. Nutr. 56, 115-122 (1986).
73. J.L. Napoli and K.R. Race, J. Biol. Chem. 263, 17372-17377 (1988).
74. N.V. Dimitrov and D.E. Ullrey in N.I. Krinsky, M.M. Mathews-Roth and R.F. Taylor, eds, Carotenoids: Chemistry and Biology, p. 269-275, Plenum Press, New York, (1990).
75. J.Ch. Meyer, H.P. Grundmann, B. Seeger and U.W. Schnyder, Dermatologica 171, 76-81 (1985).
76. J.P. Costantino, L.H. Kuller, L. Begg, C.K. Redmond and M.W. Bates, Am. J. Clin. Nutr. 48, 1277-1283 (1988).
77. E.D. Brown, M.S. Micozzi, N.E. Craft, J.G. Bieri, G. Beecher, B.K. Edwards, A. Rose, P.R. Taylor and J.C. Smith, Jr., Am. J. Clin. Nutr. 49, 1258-1265 (1989).
78. M.S. Micozzi, E.D. Brown, P.R. Taylor and E. Wolfe, Am. J. Clin. Nutr. 48, 1061-1064 (1988).
79. P. Reich, H. Swachman and J.M. Craig, New Engl. J. Med. 262, 263-269 (1960).
80. C.D. Jensen, T.W. Howes, G.A. Spiller, T.S. Patterson, J.H. Whitman and J. Scala, Nutr. Rep. Internat. 35, 413-422 (1987).
81. D.W. Nierenberg and T. Stukel, Am. J. Med. Sci. 294, 187-190 (1987).
82. R.S. Parker, Am. J. Clin. Nutr. 47, 33-36 (1988).
83. R.A. Bone, J.T. Landrum and S.L. Tarsis, Vision Res. 25, 1531-1535 (1985).
84. R.A. Bone, J.T. Landrum, L. Fernandez and S.L. Tarsis, Invest. Ophthalmol. Vis. Sci. 29, 843-849 (1988).
85. G.J. Handelman, E.A. Dratz, C.C. Reay and F.J.G.M. van Kuijk, Invest. Ophthalmol. Vis. Sci. 29, 850-855 (1988).
86. S. Singh, G.P. Mulley and M.S. Losowsky, Brit. Med. J. 297, 458-459 (1988).
87. M. Clemens, C. Ludner, G. Ehninger, H. Einsele, W. Renn, E. Buhler, H.D. Waller and R.F. Gey, Am. J. Clin. Nutr. 51, 216-219 (1990).
88. D.M. Anzo and T.A. Moore, Photochem. Photobiol. 39, 635-640 (1984).
89. R. Lee, M.M. Mathews-Roth, M.A. Pathak and J.A. Parrish, J. Invest. Dermatol. 64, 175-177 (1975).
90. A. Vahlquist, J.B. Lee, G. Michaelson and O. Rollman, J. Invest. Dermatol. 79, 94-97 (1982).
91. J.D. Ribaya-Mercado, S.C. Holmgren, J.G. Fox and R.M. Russell, J. Nutr. 119, 665-668 (1989).
92. B.S. Alam, S.Q. Alam, A. Bendich and S.S. Shapiro, Nutr. Cancer 12, 57-60 (1989).
93. R.G. Cutler, Proc. Nat. Acad. Sci. 81, 7627-7631 (1984).
94. H.R. Massie, J.R. Ferreira and L.A. DeWolfe, Gerentology 32, 189-195 (1986).
95. N.E. Craft, E.D. Brown and J.C. Smith, Jr., Clin. Chem. 34, 44-48 (1988).
96. M.M. Mathews-Roth and M.J. Stampfer, Clin. Chem. 30, 459-461 (1984).
97. D.I. Thurnham and P.S. Flora, Clin. Chem. 34, 1947 (1988).
98. H.H. Gunson, A.H. Merry, G. Britton and F. Stratton, Clin. Lab. Hematol. 6, 287-292 (1984).
99. D. Bareford, M. Cumberbatch and L.D. Tovey, Vox Sang. 46, 180-182 (1984).
100. J.N. Thompson Eur. J. Cancer Clin. Oncol. 19, 1645-1646 (1983).
101. R. Heywood, A.K. Palmer, R.L. Gregson and H. Hummler, Toxicology 36, 91-100 (1985).
102. M.M Mathews-Roth, Toxicol. Lett. 41, 185-191 (1988).
103. M.B. Poh-Fitzpatrick and R.H. Palmer, J. Lab. Clin. Med. 104, 257-263 (1984).
104. M.M. Mathews-Roth, A.A. Abraham and T.G. Gabuzda, Clin. Chem. 22, 922-924 (1976).
105. F.M. Barker, J. Toxicol.- Cutaneous & Ocular Toxicol. 7, 223-236 (1988).
106. U. Weber and G. Goerz, Klin. Mbl. Augenheilk. 188, 20-22 (1986).
107. G.B. Arden, J.O.A. Oluwole, P. Polkinghorne, A.C. Bird, F.M. Barker, P.G. Norris and J.L.M. Hawk, Human Toxicol. 8, 439-450 (1989).
108. C. Harnois, J. Samson, M. Malenfant and A. Rousseau, Arch. Ophthalmol. 107, 538-540 (1989).
109. M. Lumikari, I. Johansson, T. Ericson and J. Virtamo, Int. J. Vit. Nutr. Res. 58, 171-177 (1988).