9. Recommendations for Research

(1) Intervention studies of various types
(2) Further investigations into food composition
(3) Better biomarkers
(4) Greater understanding of the metabolism of carotenoids
(5) Better animal models
(6) Greater understanding of the mechanisms of carcinogenesis

Research proposals should clearly address gaps in knowledge about carcinogenesis and should relate to issues in carotenoid research that would clearly help answer questions relating to carcinogenesis.

Experimental research has shown that carotenoids are powerful chemicals and in

some situations can protect against cancer. As both provitamin A and non-provitamin A carotenoids have these properties, the mechanism(s) may not necessarily involve the formation of vitamin A and its biologically active metabolites. The mechanism(s) by which carotenoids inhibit carcinogenesis in experimental animals is not known.

Carotenoids can suppress cancer growth and development when they are applied to epithelial surfaces, when they are given orally and when injected into tissues. It is important to determine the uptake, transport and metabolism of the carotenoids in each case.

The natural site for carotenoids is membranes, where they affect the properties and those of associated processes and therefore potentially affect mechanisms of carcinogenesis. Research is required on caro-tenoid–cell membrane interactions in these situations and whether they are relevant to the prevention of cancer in humans.

Retinoids are clearly important in controlling cell differentiation and tissue growth. The morphogen, retinoic acid, can be formed directly from β-carotene in some circumstances. Central cleavage of carotenoids has been reported in many tissues, and there is evidence that the turnover of the provitamin A carotenoids β-carotene, α-carotene and β-cryptoxanthin is more rapid than that of the other major carotenoids in serum, suggesting conversion to retinol. Research should be conducted on the effects of carotenoids and carotenoid metabolites on events mediated by retinol receptors. This potential conversion could be studied by analysing endogenous responsive genes or by use of reporter constructs, which would allow the detection of activity ligands at levels as low as 10^{-10} mol/L.

Carotenoids are powerful quenchers of singlet oxygen and triplet sensitizers, a property that is relevant to the treatment of erythropoieticprotoporphyria. Furthermore, carotenoids can act as either antioxidants or pro-oxidants in chemical systems. The popular hypothesis that any biological activity of carotenoids can be attributed to an antioxidant or pro-oxidant role has not been proved. Research is required to evaluate critically the postulated antioxidant action of carotenoids *in vivo*, i.e. protection against oxidation mediated by free radicals. Interactions between carotenoids and vitamins E and C and other intracellular antioxidant defence mechanisms should also be studied.

There is accumulating evidence that metabolites of some of the common dietary carotenoids are produced oxidatively in biological fluids. Little is known about the basic mechanisms of oxidation of carotenoids or about reactions with free radicals. Research is required to understand the basic oxidative mechanisms that affect carotenoids *in vivo* in order to appreciate if and when carotenoids are influenced by such stresses.

Oxidative stress is a powerful signal (stimulator) of immune mechanisms. The operation of such mechanisms may be evident many years before clinical symptoms of disease appear; for example, a small depression in negative acute-phase proteins can be detected retrospectively 5–10 years before the appearance of clinical disease. Carotenoids are very sensitive to oxidation, and the presence of detectable oxidation products could be a useful marker of oxidative stress and of disease for use in epidemiological studies. Oxidation products of carotenoids may themselves have biological activity, and conversion within target tissues could explain much of their action. Research is needed to identify products of oxidation and metabolism and to examine their potential activity.

Major problems in assessing the cancer-preventive properties of carotenoids and in evaluating their adverse effects include the issues of antioxidant activity and their pro-oxidant role and the effects on lung cancer as related to cigarette smoking. The following research areas may be recommended:

- Development and application of suitable animal models for evaluating the oxidant and antioxidant properties of carotenoids *in vivo* and modulation of cigarette smoke-related biomarkers in the respiratory tract and cardiovascular system
- Implementation of phase-II trials to evaluate the oxidative and antioxidant effects of carotenoids and modulation of cigarette

smoke-related biomarkers in cells of the respiratory tract, e.g. pulmonary alveolarmacrophages.

Intervention studies with high doses of β-carotene provide evidence that people who are current smokers are at increased risk for lung cancer and cardiovascular disease when given supplements of β-carotene. Research is needed on the metabolic effects that smoking has on tissue metabolism and human physiology and on the effect of tobacco smoke on carotenoids *in vivo*.

Smoking is clearly associated with lower serum levels of some carotenoids. Research is required to determine whether this occurs through effects on the diet or on metabolism. Studies to determine the effects of tobacco smoke on carotenoids and of the interaction of carotenoids with smoke-exposed tissues should be conducted to better understand why β-carotene had the effect it did and whether this effect is unique or is common to all carotenoids.

Most of the observational studies were unable to distinguish the effects of individual carotenoids from those of substances in fruits and vegetables. Refinement of dietary databases, repetition of studies with adequate methods for evaluating diets and new biomarkers of intake should be pursued. Another approach is to capitalize on the fact that human populations differ in their intake of carotenoids. Research in regions where subpopulations have high intakes of specific carotenes deserves high priority.