9. Recommendations for future research

The assessment of the literature presented in this handbook led to formulation of the following recommendations for future research:

1. To study the mechanism of action of retinol and its metabolites at the molecular and genetic levels in relation to carcinogenesis;

2. To identify new nontoxic conjugates and formulations of retinol and retinyl esters that may show cancer-preventive properties;

3. To consider the conduct of randomized prevention trials of longer duration among particularly high-risk groups such as asbestos-exposed individuals and former lung cancer patients;

4. To evaluate exposure biomarkers useful in epidemiological studies and clinical trials;

5. To continue the follow-up of the terminated intervention trials to assess the long-term effects of the intervention agents.

It is becoming clear that the effects of retinol metabolites such as the retinoic acids on their nuclear receptors are modulated, either directly or indirectly, by other important nuclear receptors and their ligands (glucocorticoids, estrogen, thyroid hormones, vitamin D and prostaglandins), other transcription factors and intermediary proteins. The retinoid signalling system is even more complex in that there are at least two classes of retinoid receptors and three subtypes of each class that in the presence of their ligands can activate or repress retinoic acid responsive elements on genes. Depending on the dimeric partner and response element, receptors can be affected differently by their natural or synthetic ligands. Thus, much remains to be learned on the molecular mechanisms controlling these processes, the interaction of the different pathophysiological factors involved at cellular and tissue levels, and how such environmental factors as asbestos, tobacco, bidi smoking, betel nut chewing, alcohol and environmental toxins influence these systems.

A better understanding of the way the cell and the body control their vitamin A supply will allow better definition of vitamin A status, distribution at times of deficiency, and assessment of effects on how distribution is organized. Epidemiologically, the eye has been considered the organ most vulnerable to vitamin A status. However, in the last decade, the importance of vitamin A for immune function has become apparent. Impaired immune function has been identified in populations where ophthalmological signs are rare, and morbidity is reduced by vitamin A supplements. Animal studies have shown that vitamin A deficiency increases susceptibility to chemical carcinogens. Thus, the aspects of vitamin A metabolism that are disrupted during deficiency should be identified, as should the point at which vitamin A depletion occurs. Other questions that still need to be resolved are how adaptation to low vitamin A intake occurs and what pathway is attenuated or blocked so that vulnerability to experimental carcinogenesis is enhanced.

In some situations, different sources of vitamin A (retinol, retinyl palmitate and retinyl acetate) appear to have different effects *in vivo* and comparative experiments should be conducted to investigate such observations in well defined experimental models. Concern was expressed that combinations of other micro-nutrients with vitamin A, as well as foods, might influence retinoid metabolism. These factors should also be studied at the cellular level and include metabolites that might arise under experimental conditions, where large doses are given.

Much current research has been directed to explaining how retinol and other retinoids function at the genetic level. Responses of genes to retinoids controlling cellular differentiation and errors in such control can determine cancer risk. Genetic diversity in human populations means that certain genetic polymorphisms within the population may predispose to cancer. Individuals who respond positively to increased intake of vitamin A or to

modifications in retinoid metabolism should be identified.

It is difficult to evaluate and compare results of studies in which protocols differed widely or in which proper controls and differences in the baseline conditions were neglected, omitted or inadequately described. There is an urgent need to develop standard protocols to evaluate the cancer-preventive properties of retinol and retinoids. Standard protocols should specify continuous quality control of supplements and the type of formulation to be used. Such a strategy should be developed in all studies: molecular, cellular, animal and human. Although standardization in human studies is probably the most difficult, these protocols would help to identify, minimize or quantify the variability that undoubtedly exists in human studies.