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Relative value of carotenoids as precursors of vitamin A

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The carotenoid pigments are very conspicuous in nature, owing to their yellow to red hues, and occur without exception in photosynthetic tissue. They are responsible for the colour in some species of yeast, bacteria and fungi as well as many vegetables and fruits. Although animals are incapable of *de novo* synthesis, they are able to deposit the carotenoid pigment as absorbed or with some alteration of the basic structure. Thus, the red to yellow colour in the flesh, skin, shell or exoskeleton of salmon, lobster, crab, prawn, carp, flamingo, etc. is directly or indirectly diet-related. The intestinal cleavage of carotenoids to form vitamin A active retinoids represents the main contribution of the carotenoids to nutrition. The colour associated with foods such as vegetables, fruits, butter, egg yolks, salmon, etc. represents an aesthetic contribution made by these pigments.

An inspection of other papers in this symposium demonstrates that the retinoids have a wider application to health than has been traditionally thought. Likewise, recent findings support the suggestion for a wider, non-vitamin A role of the carotenes.

The structures of some common carotenoids are shown in Fig. 1. β-Carotene, while not the most abundant carotene is, nevertheless, very widespread in nature. Both halves of β-carotene are related to retinol, thus the compound possesses maximal provitamin A activity. γ-Carotene, with one ring, α-carotene with a 4′,5′ double bond in the ring, and β-cryptoxanthin (xanthophyll) and β-carotene-5,6-epoxide, with substituted rings, contribute 50% of the activity of β-carotene.

Biosynthesis

Recent reviews have been published which detail the various aspects of the biosynthesis of carotenoids (Britton, 1976; Davies, 1979; Goodwin, 1981; Simpson *et al.* 1981). Key compounds in the terpenoid synthesis are acetyl CoA and the 6-carbon compound, mevalonic acid. Isopentenyl pyrophosphate serves as a 5-carbon unit leading to the 40-carbon compound, phytoene. Successive dehydrogenations and cyclizations result in compounds such as β-carotene and subsequent oxygenations lead to the xanthophylls.

Changes in the accumulation of carotenoids in plants have been made through genetic breeding, storage, environmental factors and bioregulators (Simpson & Chichester, 1981).

Stability

The carotenoids are very unstable because of their conjugated system of double bonds. Degradative changes of provitamin A compounds in foods during processing and cooking result in the lowering of vitamin A activity (Simpson *et al.*
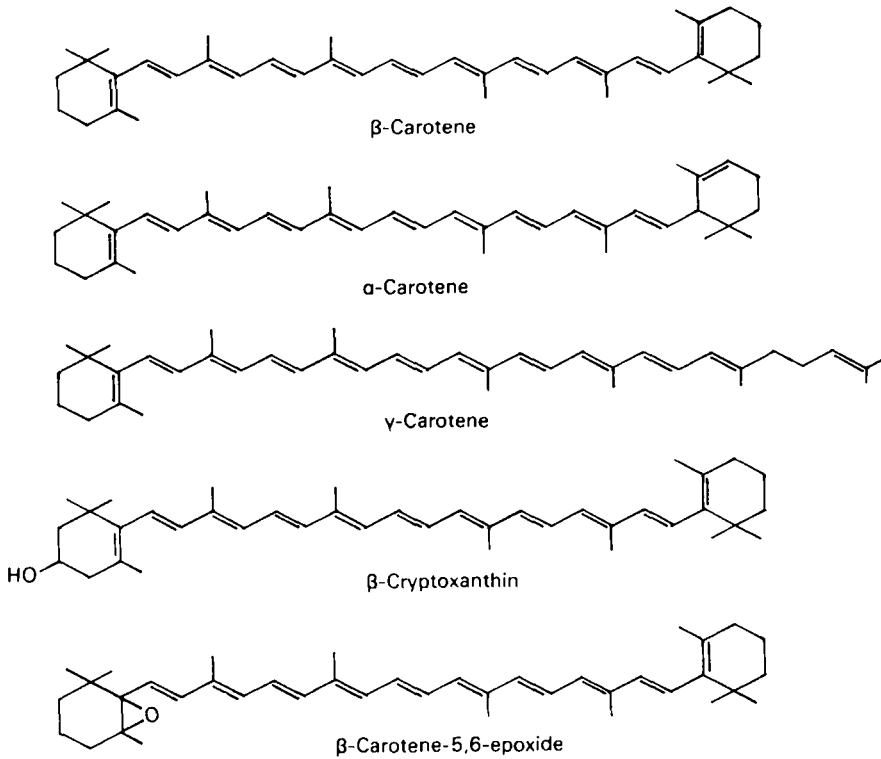
Fig. 1.   Structures of some common carotenoids.

1976; Simpson & Chichester, 1981). In general, the carotenoids are destroyed or altered by acids, particularly in the presence of light, to form *cis* isomers from the all *trans* structure. These pigments are easily oxidized in the presence of oxygen, catalyzed by fluorescent light and lipoxygenase, commonly with the co-oxidation of unsaturated fatty acids. The initial site of attack is the 5,6 double bond or in-chain double bonds leading to epoxide formation and chain cleavage.

Drying and extrusion cooking are particularly destructive processing steps, whereas cooking or canning generally result in only slight losses. Certain cooking methods can significantly lower provitamin A content. Sood & Bhat (1974) compared the traditional (Indian) method in which vegetables are boiled in open vessels until the water is evaporated, to other methods of vegetable cooking. The traditional method gave poor retention of carotene. Other studies have shown that cooking losses are minimized where processing time and temperature are kept low (Rao & Reddy, 1979).

Provitamin A compounds

A number of authors have listed provitamin A active carotenes and retinoids (Olson & Lakshmanan, 1970; Bauernfeind, 1972; Singh & Cama, 1975; Sporn *et al.* 1976). Bauernfeind (1972), for example, lists thirty-two carotenoids and apo-carotenals with provitamin A activity. Since the number of known carotenoids

Table 1.   *Representative types of carotenoids and apocarotenoids with provitamin*
*A activity**

Carotenoids	Activity (%)
β-Carotene	100
Neo-β-carotene U	38†
α-Carotene	50–54
Neo-α-carotene U	13†
3,4,-Dehydro-β-carotene	75
3,4,3′,4′-Bisdehydro-β-carotene	38
γ-Carotene	42–50
7,8′-Dihydro-γ-carotene	20–40
β-Carotene-5′,6′-epoxide	21
α-Carotene-5,6-epoxide	25
β-Carotene-5,6,5′,6′-diepoxide	Active
3-Keto-β-carotene	52
3-Hydroxy-β-carotene	50–60
4-Hydroxy-β-carotene	48
β-Apo-2′-carotenal	Active
β-Apo-8′-carotenal	72
β-Apo-10′-carotenal	Active
β-Apo-12′-carotenal	120
Lycopene	Inactive
Lutein	Inactive

*From Baurenfeind (1972).
†From Zechmeister (1949).

now exceeds 500, it can be seen that the vitamin A active compounds are only a fraction of the total. Table 1 lists some representative compounds from Bauernfeind's list.

Changes in the structure of β-carotene generally result in lowered activity. Vitamin A activity has been assigned on the basis of animal bioassay, in vitro methods using β-carotene 15,15′-dioxygenase (*EC* 1.13.11.21) with the formation of retinal, or 'inspection' of the molecule for presumed activity. On the basis of the latter 'technique', the list of provitamin A active compounds approaches sixty. The list, however, condenses to about five or six pigments commonly found in food (see Fig. 1).

Zechmeister (1949) lists the relative biological potency of β-carotene, α-carotene, γ-carotene, cryptoxanthin and some *cis–trans* isomers of these compounds. As expected, the *cis* isomers are less active. Sweeney & Marsh (1973) gave rats *cis–trans* isomers of β- and α-carotene and found an enrichment of the all *trans* structures in the faeces, suggesting a geometric isomerization in the acid environment of the stomach.

Absorption and utilization

During digestion the carotenoids in food are subjected to the action of esterases, lipases and proteases which releases the pigments to be solubilized by the bile salts. Within the mucosal cell, the carotenes may be oxidatively cleaved to retinoids or,

to a lesser degree, absorbed intact. The conversion to retinol is not an efficient process. It is generally recognized that, in terms of absorption, 1 μg retinol is equivalent to 6 μg β-carotene and 12 μg of other carotenoids. The actual value could be higher or lower depending on the level of dietary lipid (Jayarajan *et al.* 1980), protein (Kamath & Arnrich, 1973), other carotenes (Premachandra *et al.* 1976) and bile salts (El-Gorab *et al.* 1975).

The value of the carotenoids in the formation of retinol is directly related to the nature of the enzymatic cleavage. It has been proposed that β-carotene is specifically, oxidatively cleaved to yield two molecules of retinal.

A series of papers have reported the isolation of a specific β-carotene 15-15′-dioxygenase (Goodman *et al.* 1966; Lakshmanan *et al.* 1968; Olson, 1968; Lakshmanan *et al.* 1972; Singh & Cama, 1974) from several different animals. The enzyme was inactive on mono- and diepoxides (Lakshmanan *et al.* 1968). Although the epoxides are provitamin A active, it is possible that the epoxide is converted to the parent hydrocarbon during the in vivo digestion process.

A second mechanism has been proposed in which β-carotene is eccentrically cleaved to yield apo-carotenals which are degraded in a stepwise manner to retinal. Sharma *et al.* (1977) gave β-carotene to either rats or chickens and isolated retinal and the 8′, 10′ and 12′-β-apo-carotenals. When the 8′-β-apocarotenal was given, the shorter 10′ and 12′ compounds were isolated. Sharma *et al.* (1977) proposed that the specificity of the enzyme(s) involved is mainly toward the 15-15′ cleavage, but significant splitting, which in some cases is stepwise, occurs at the 12′, 10′, 8′ positions to yield the corresponding apo-carotenals. These in turn may be split to shorter apo compounds and retinal, or oxidized to the acid leading to retinoic acid.

It is very difficult to draw conclusions from these results. It seems probable that the application of high pressure liquid chromatography (HPLC) to the products of the '15-15′ cleaving enzyme' might yield additional products. It is also possible that further enzyme purification might yield enzymes with different positional specificities.

The central cleavage of α-carotene has been found to result in retinol and α-retinol (McAnally & Szymanski, 1966). α-Retinol does not combine with retinol-binding protein (RBP) (Muhilal & Glover, 1975), and thus is capable of inducing hypervitaminosis A. α-Retinol functions as vitamin A at the tissue level but it does not combine with RBP and shows a negative result in animal bioassays (Pitt, 1978). Central cleavage of xanthophylls and acyclic, epoxide and keto-carotenoids might also yield retinoids that could be active if they could be transported to the tissues. The biological activity of 5,6-epoxyretinoic acid has been shown to vary from 157 to 0·5% of the activity of retinyl acetate for growth promotion in rats (Zile *et al.* 1980). These authors do not rule out the possibility that this compound is not transported to the tissue and hence has no apparent role.

The efficiency of the cleavage of carotenes has a direct bearing on the level of plasma carotenoids and tissue storage. The rat intestine has a very efficient cleavage enzyme which results in low plasma carotenoid levels, whereas the human intestine is less efficient and thus some plasma carotenoids can be found. Once

absorbed, the carotenoids may be deposited in the liver or other organs, fat depots, adrenal cortex, atheromatous plaques, flesh, skin, milk and eggs (Simpson & Chichester, 1981). A high consumption of carotenoids has resulted in skin pigmentation in the treatment of erythropoietic protoporphyria and the condition of lycopenaemia and carotenaemia (Simpson & Chichester, 1981). It appears that at least the liver (Olson & Hyaishi, 1965) and bovine corpus luteum (Gawienowski *et al.* 1974) contain cleavage enzymes to split absorbed carotene.

The carotenoids have long been known to play a protective role in green plants (Krinsky, 1979). Recently, epidemiological information has accumulated suggesting a possible physiological role for the carotenoids apart from their role as precursors to retinol. The so-called 'Western Electric Study' (Shekelle *et al.* 1981) compared the incidence of lung cancer in middle-aged men to their consumption of carotene and retinol. This 19 year study found an inverse relationship between the consumption of carotene and the incidence of lung cancer in both smokers and non-smokers. Carotene and retinol consumption was not significantly related to other carcinomas nor was retinol found to be a factor in lung cancer prevention. The study assumes, however, that the active factor in a diet rich in vegetables and fruit is carotene. The study (and especially the news release) included fruits and vegetables that are actually devoid of carotene or are poor sources of it (e.g. beets, cauliflowers, aubergines, apples, etc.).

The possible relationship between cancer and β-carotene has been elegantly reviewed by Peto *et al.* (1981). These authors conclude that 'Although the evidence thus far available is not compelling that β-carotene is truly protective against cancer (or *a fortiori* against total mortality), this is not a good reason to delay starting controlled trials because even if, as seems probable, a few truly protective agents do await discovery among the dozen or two dietary factors of current interest to the research community, compelling evidence may take decades to emerge without controlled trials.'

In unpublished work, Gerber and Erdman found that the wounds of rats given β-carotene healed faster than those given retinyl acetate. These workers speculated that the conversion of β-carotene to a form of vitamin A which was more potent in wound healing, either intestinally or extra-intestinally, is possible, or alternatively, perhaps β-carotene itself has some function in the wound-healing process.

Our thinking has consistently focused on β-carotene because of its vitamin A role. However, many of the foods surveyed in the Shekelle *et al.* (1981) study contained low levels of β-carotene in relation to other carotenoids (e.g. tomatoes 10%, green tissue 20%, egg yolk 5%). The significance of these other carotenoids in the human diet has not yet been assessed.

Methods of analysis of provitamin A carotenes

All methods of carotenoid analysis depend on an initial extraction often followed by saponification and chromotography. The result of the analysis may be the quantification of individual pigments or some grouping of pigments into fractions. The accurate determination of provitamin A content depends on the separation of

provitamin A active and inactive compounds, however this is not always done. The reason, of course, is that the complete separation requires several hours whereas a partial separation may take much less time and effort. The much more simple Association of Official Analytical Chemists (AOAC) (1980) type method chromatographically separates the carotenes from the oxygenated compounds. It does not separate individual carotenes, cis–trans isomers or carotenoid esters. The estimate is good if β-carotene is the only provitamin A compound and the remaining pigments are polar. The estimation is poor where the compounds in the tissue have similar polar and light-absorbing characteristics to β-carotene.

Despite the fact that HPLC analysis is relatively new, it has been demonstrated that the method is rapid, reproducible, and thus creates few artifacts. HPLC analysis may be classified on the basis of the adsorbent and solvent systems employed. Carotenoids have thus been separated by isocratic and gradient solvent systems on normal- or reverse-phase adsorbents.

Isocratic solvent system on normal-phase adsorbent. Reeder & Park (1975) used two columns and two isocratic solvent schedules to separate α-carotene, β-carotene and cryptoxanthin. Table 2 shows their provitamin A analysis for orange juice. If one calculates the retinol equivalents on the basis of their separation results, it can be seen that the traditional AOAC method overestimates the provitamin A content by a factor of two.

Gradient elution and normal-phase adsorbent. A number of papers have reported separations by this method using primarily silica and magnesium oxide columns. The method does separate carotenes and xanthophylls but also requires a long equilibrium period to recondition the column. A good separation of α- and β-carotene was not reported using these conditions (Fiksdahl, 1978).

Reverse phase and isocratic solvent or gradient elution system. The reverse-phase system would be expected to produce fewer artifacts because of the relatively weaker bonding forces involved. This was proven to be the case by Braumann & Grimme (1981). When they chromatographed the compounds on silica and then rechromatographed them on reverse phase, they found significant artifact production from the silica. The choice between isocratic and gradient elution depends on the polar variation between the compounds to be separated

Table 2. *Comparison of provitamin A analyses (µg/ml) in valencia orange juice**

Method...	AOAC		HPLC				
Sample	Total carotene	Retinol† equivalent	α-Carotene	β-Carotene	Crypto-xanthin	Total carotene	Retinol† equivalent
A	1·86	0·31	0·15	0·23	1·22	1·60	0·15
B	2·14	0·36	0·13	0·20	1·08	1·41	0·16
C	1·60	0·27	0·11	0·14	1·06	1·31	0·14

AOAC, Association of Official Analytical Chemists.
HPLC, high pressure liquid chromatography.
*From Reeder & Park (1975).
†Our calculation.

(carotene and/or xanthophylls). The isocratic, reverse-phase system has been reported to separate α- and β-carotene (Zakaria *et al.* 1979).

The gradient solvent system reported by Braumann & Grimme (1981) (linear gradient methanol:acetonitrile (25:75) water (100) solvent system) separated the chloroplastic xanthophylls and chorophyll, and α- and β-carotene.

A long equilibrium period may be required with gradient elution if reproducible retention times are to be achieved. The RCM-C18 5 μm cartridge, eluted with a gradient solvent system of water in methanol (3:97) to tetrahydrofuran (THF) in methanol (10:90), resolved most carotenoids in 30 min, reduced the back washing time to 10 min and gave reproducible retention times (Table 3) (Tsou and Simpson, unpublished results). The same RCM-C18 column run isocratically with a solvent system of THF in methanol (10:90), separated cryptoxanthin, lycopene, α-carotene and β-carotene in 17 min.

A number of authors, using HPLC as well as open column techniques, have pointed out the unreliability of the food composition tables. Gebhardt *et al.* (1977) compared the AOAC method and open column techniques for the separation of clingstone peach carotenoids. The AOAC method gave an overestimation for both the raw (60% of recommended dietary allowance (RDA) *v.* 11%) and canned (12% *v.* 6%) products. C. Y. Lee (personal communication) reported a twofold overestimation for carrots and Zakaria *et al.* (1979) a greater than tenfold overestimation for tomatoes. Because of the capabilities of the instrumentation at the time of the development of the AOAC method, 436 nm was used as the measuring wavelength.

Table 4 shows the comparison of the provitamin A values for spinach based on the separation of β-carotene (Yang, Tsou and Simpson, unpublished results). Table 4 points out several areas of concern. The results show that without interfering-carotenes, as in spinach, HPLC and AOAC separations are similar. Some error is introduced with the AOAC calculations. Variety, maturity or handling are variables that may account for the 100% variation for spinach analysed by the same method.

Table 3. *Retention times (min) of selected carotenoids separated by RCM-C18 cartridge with gradient elution**

Injection	Neoxanthin	Violaxanthin	Lutein	β-Carotene
1	3·45	4·24	7·42	26·5
2	3·46	4·26	7·47	26·7
3	3·48	4·27	7·49	26·9
4	3·46	4·25	7·46	26·6
5	3·48	4·28	7·51	26·7
Mean	3·47	4·26	7·46	26·7
Standard deviation	0·01	0·02	0·04	0·1

Solvent gradient: A: H_2O:MeOH (3:97)
B: THF:MeOH (10:90)
0 to 100% B 10 min curve 8
10 min re-equilibration with solvent A between runs.
*From Tsou and Simpson, unpublished results.

Table 4. *Comparison of provitamin A values in spinach*

Method of analysis	β-Carotene	
	Retinol equivalent (μg/g)	i.u./g
HPLC separation	10·09	101
AOAC*	10·0	100
AOAC†	14·0	140
Adams (1975)	7·9	79

HPLC, high pressure liquid chromatography.
AOAC, Association of Official Analytical Chemists.

*Measurement at 450 nm $E_{1\ cm}^{1\%}$ 2600.

†Measurement at 436 nm $E_{1\ cm}^{1\%}$ 1960 (cf. AOAC, 1980).

In Table 5, fruits were selected from the listing of Goodwin & Goad (1970) and data of Adams (1975) were calculated to give β-carotene values. Fruit normally containing anthrocyanin-type pigments (cherry) are listed as having a high β-carotene content. The β-carotene contents of avocado, apricot, papaya, musk melon and red watermelon are overestimated mainly because compounds other than β-carotene are included. The tables of carotene values listed by Goodwin & Goad (1970) and Adams (1975) show great variability and thus reflect the fact that they are composed of compilations by a large number of authors who used several methods and different samples. The tables, in turn, are used by nutritionists who often accept the information without reservation. For example, the 'Western Electric Study' (Shekelle *et al.* 1981) included vegetables and fruits which are poor sources of provitamin A.

Table 5. *Total carotenoids and β-carotene contents (mg/kg fresh wt) of selected fruits and vegetables*

	Total carotenoid*	β-Carotene*	β-Carotene equivalent**
Apricot	35	21	69†
Avocado	5·6	0·4–0·5	3·9†
Watermelon			
Orange	33·7	1·4	0·1‡
Red	20·9–61·7	0·41–5·96	1·4‡
Musk melon	20	17·1	108†
Japanese persimmons	21·6–97·9	—	6·0‡
Pepper			
Red	127–248	11·6–33	43·8†
Green	9·0–11·2	1·2–1·5	4·1†
Mango	13–62	4·77–39·9	66·5†
Cherry	——	5–11	5·4†
Papaya	13·8	4·1	7·0†

*Isolation method from Goodwin & Goad (1970).
**Calculated from i.u. vitamin A.
†AOAC method in Adams (1975).
‡AOAC method in Leung *et al.* (1968).

Intervention strategies

The source of vitamin A from preformed retinol and/or carotene varies depending on the culture. Recent statistics show that carotene from vegetables contributes 68% of dietary vitamin A on a world-wide basis and 82% in developing countries. In spite of the abundance of carotene in the world, vitamin A deficiency is still a very serious problem. One consequence of vitamin A deficiency, nutritional blindness, claims an estimated half million victims each year; in Indonesia alone an estimate of 60 000 cases has been reported (Anon, 1980). The problems associated with an inadequate dietary intake of vitamin A in other areas would include unavailability of vegetables during a dry season, poor cooking methods (boiling *v.* stir frying), diet composition (lack of oil), poor economic conditions (selling mangoes, eggs, etc. rather than eating them) and cultural habits.

Two examples may illustrate the point that some cultural practices may cause problems: the bitter melon (charantia fruits) in Southeast Asia is always consumed at the mature green stage when the β-carotene content is low rather than at the fully ripe stage when the carotene content is high; also, a familiar saying in a part of Indonesia translates, 'A goat eats grass and I eat the goat . . . why should I eat grass also?'

Intervention strategies based on retinol administration have been very effective in selected areas and population segments (e.g. during parasitic infestation, infection and/or diarrhoeal diseases, and in diet supplements given to lactating women). The promotion of dietary intake of provitamin A from fruits and vegetables and the promotion of conditions for maximum intestinal retention of the vitamin A precursors would seem to be the only practical solution for the developing countries.

A single programme for the seventy-four countries listed as 'at risk' is not possible, but the elements of a programme should include the following:

1. Using β-carotene content as a quality characteristic in plant-breeding programmes.
2. Promoting preservation and distribution practices based on preventing loss of carotenes.
3. Revising the vitamin A composition tables in the nutritional data books to reflect new values.
4. Promoting studies aimed at altering intestinal absorption of dietary carotenes without substantially altering cultural practices.
5. Initiating extension projects based on demonstration garden plots to increase the effective consumption of provitamin A.

The Asian Vegetable Research and Development Center initiated a home and garden programme in 1980 (Gershon, 1982). The home-garden plots were designed to be small (4×4 m) and to grow crops which were culturally acceptable and nutritionally oriented. The goal was to meet the following percentages of the RDA for a 10–12 year old child: calcium 40, iron 40, vitamin A 80, vitamin C 100.

Table 6. *Percentage contribution to the recommended dietary allowance for a family of five: expected versus observed results, home gardens*[*]

	Protein	Calcium	Iron	Vitamin A	Vitamin C
Expected ...		40	40	80	100
Observed					
Thailand	19	67	75	104	633
Indonesia	23	51	74	126	254
Philippines	6	12	26	22	91
'Vitamin A garden'	15	56	83	124	492

[*]Gershon (1982).

The garden plots were developed from the low-land tropics for a family of five in the Philippines, Indonesia and Thailand. Vegetables and fruits were chosen that were consumed by a typical family. Table 6 provides a summary of the results of the first 4 month period. Generally, the gardens in Thailand and Indonesia performed well. Aubergine, which is a popular vegetable in the Philippines, was included in that country's gardens even though it contributed little to the nutrition targets. The results show that technically a small plot could provide sufficient vegetables to make a significant nutritional contribution to the diet of a small family. The technology is the easy part whereas the widespread use of such a garden will require some effort.

REFERENCES

Adams, C. F. (1975). *Nutritive Value of American Foods in Common Units*. Agricultural Handbook no. 456 ARS. Washington DC: USDA.
Anon. (1980). *Indonesian Nutritional Blindness Prevention Project: Characterization of Vitamin A Deficiency and Xerophalmia and the Design of an Effective Intervention Program*. Jakarta: Division of Nutrition, Ministry of Health, Republic of Indonesia and Helen Keller International.
Association of Official Analytical Chemists (1980). *Official Methods of Analysis,* 12th ed. Washington DC: AOAC.
Bauernfeind, J. C. (1972). *J. agric. Fd Chem.* **20,** 456.
Braumann, T. & Grimme, L. H. (1981). *Biochim. biophys. Acta* **637,** 8.
Britton, G. (1976). *Pure appl. Chem.* **47,** 223.
Davies, B. H. (1979). *Pure appl. Chem.* **51,** 623.
El-Gorab, M. L., Underwood, B. A. & Loerch, J. D. (1975). *Biochim. biophys. Acta* **401,** 265.
Fiksdahl, A., Mortensen, J. T. & Liaaen-Jensen, S. (1978). *J. Chromat.* **157,** 111.
Gawienowski, A. M., Stacewicz-Sapuncakis, M. & Longley, R. (1974). *J. Lipid Res.* **15,** 375.
Gebhardt, S. E., Elkins, E. R. & Humphrey, J. (1977). *J. agric. Fd Chem.* **25,** 628.
Gershon, J. (1982). *AVRDC Garden Program Technical Report*. Tainan, Taiwan: Asian Vegetable Research and Development Center.
Goodman, D. S., Huang, H. S. & Shiratori, T. (1966). *J. biol. Chem.* **241,** 1929.
Goodwin, T. W. (1981). *The Biochemistry of the Carotenoids*. Vol. 1. *Plants*. London and New York: Chapman Hall.

Goodwin, T. W. & Goad, L. J. (1970). In *The Biochemistry of Fruit and their Products,* vol. 1, p. 305 [A. C. Hulme, editor]. London and New York: Academic Press.

Gross, J. & Budowski, P. (1966). *Biochem. J.* **101,** 747.

Jayarajan, P., Reddy, V. & Mahanram, M. (1980). *Ind. J. med. Res.* **71,** 53.

Kamath, S. K. & Arnrich, L. (1973). *J. Nutr.* **103,** 202.

Krinsky, N. I. (1979). *Pure appl. Chem.* **51,** 649.

Lakshmanan, M. R., Chansang, H. & Olson, J. A. (1972). *J. Lipid Res.* **13,** 477.

Lakshmanan, M. R., Pope, J. L. & Olson, J. A. (1968). *Biochem. biophys. Res. Commun.* **33,** 347.

Leung, W-T. W., Butrum, R. R., Chang, F. H., Narayana, R. & Polacchi, W. (1972). *Food Composition Table for use in East Asia.* Washington DC: DHEW.

McAnally, J. C. & Szymanski, C. D. (1966). *Nature, Lond.* **210,** 1366.

Muhilal, H. & Glover, J. (1975). *Biochem. Soc. Trans.* **3,** 744.

Olson, J. A. (1968). *Am. J. clin. Nutr.* **22,** 953.

Olson, J. A. & Hayaishi, O. (1965). *Proc. natn. Acad. Sci. USA* **54,** 1364.

Olson, J. A. & Lakshmanan, M. R. (1970). In *Fat Soluble Vitamins,* p. 213 [H. F. DeLuca and J. W. Suttie, editors]. Madison, Wisconsin: University of Wisconsin Press.

Peto, K., Doll, R., Buckley, J. D. & Sporn, M. B. (1981). *Nature, Lond.* **290,** 201.

Pitt, G. A. J. (1978). *Wld Rev. Nutr. Diet.* **31,** 65.

Premachandra, B. R., Vasantharajan, V. N. & Cama, H. R. (1976). *Curr. Sci.* **45,** 56.

Rao, S. & Reddy, U. M. (1979). *Proc. 1st Indian Conv. Food Sci. Tech.* **11,** 12A871.

Reeder, S. K. & Park, G. Y. (1975). *J. AOAC* **58,** 595.

Sharma, R. V., Mathur, S. N., Dmitrovskii, A. A., Das, R. C. & Ganguly, J. (1977). *Biochim. biophys. Acta* **468,** 183.

Shekelle, R. B., Liu, S., Raynor, W. J. Jr, Lepper, M., Maliza, C. & Rossot, A. H. (1981). *Lancet* ii, 1185.

Simpson, K. L. & Chichester, C. O. (1981). *Ann. Rev. Nutr.* **1,** 351.

Simpson, K. L., Katayama, T. & Chichester, C. O. (1981). In *Carotenoids as Colorants and Vitamin A Precursors,* p. 463 [J. C. Bauernfeind, editor]. London and New York: Academic Press.

Simpson, K. L., Lee, T-C., Rodriguez, D. B. & Chichester, C. O. (1976). In *Chemistry and Biochemistry of Plant Pigments,* vol. 1, p. 780 [T. W. Goodwin, editor]. London and New York: Academic Press.

Singh, H. & Cama, H. R. (1974). *Biochim. biophys. Acta* **370,** 49.

Singh, H. & Cama, H. R. (1975). *J. Sci. Inc. Res.* **34,** 219.

Sood, R. & Bhat, C. M. (1974). *J. Fd Sci. Tech.* **11,** 131.

Sporn, M. B., Dunlop, N. M., Newton, D. L. & Henderson, W. R. (1976). *Nature, Lond.* **263,** 110.

Sweeney, J. P. & Marsh, A. C. (1973). *J. Nutr.* **103,** 20.

Zakaria, M., Simpson, K. L., Brown, P. & Krstulovic, A. (1979). *J. Chromat.* **176,** 109.

Zechmeister, L. (1949). *Vitams Horm.* **7,** 57.

Zile, M. H., Inhorn, R. C. & DeLuca, H. F. (1980). *J. Nutr.* **110,** 2225.