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SYMPOSIUM ON THE CHEMICAL ASPECTS OF NUTRITION NEEDS

Introduction

We are now in the middle of a dynamic period of transition in modifying nutritional attitudes toward man's welfare. The major micronutrients have been already identified as vitamins and mineral elements. Furthermore, the major macronutrients, so long recognized as carbohydrate, protein, and fat, are now being redefined. For instance, at least in animal nutrition, we conceptualize amino acid requirements of the various species rather than protein needs.

I say a transition period because man is now adopting animal feeding principles from "down on the farm" and is applying these to feeding his own species. For instance, while lysine-supplementation of flour was suggested by a very few farsighted men 20 years ago, it is now being considered quite favorable in many countries—although it is not yet in common use. Also, five additional vitamins and three additional minerals have been added to the list of nutrients in the Recommended Dietary Allowances. The restoration enrichment principle for flour may soon give way toward a more logical and comprehensive nutrification program in order to make flour a nutritional basic food, rather than just relatively empty calories.

All of these relationships will be presented in this symposium by men who are recognized internationally not only for their original research achievements in the field of nutrition, but also for their eloquence of thought and presentation in placing their findings into the context of contemporary world nutritional problems.

New developments in the structure or metabolic function of vitamin and amino acid nutrients or to their deficiency states in man will be presented. All relate to man and his nutrient needs, although some will describe modified requirements to overcome recently discovered genetic defects often revealed by unique forms of metabolic disease. Increased vitamin needs at the tissue level under certain conditions of air pollution will be suggested. It will be shown, too, that certain metabolic changes resulting from taking oral contraceptive steroids can be largely normalized by administering a dose level of one of the B-group of vitamins which is much greater than can be attained by diet alone. Furthermore, special efforts will be made to focus on human amino acid nutrition.

Some reports concern domestic and foreign problems in human nutrition. Practical solutions will be presented along with data on nutritional intervention techniques which proved efficacious under field conditions. In addition, extrapolations will be made toward anticipating human feeding problems 50 years hence.

Some papers have been included because of their interdisciplinary, *i.e.*, medical vs. agricultural, interest and others for the provocative or nontraditional modes of thought which the investigator has applied to solving current nutritional problems. All will speak from the conviction of their own research careers based upon extensive experience from within and from outside of the research laboratory. Hopefully differences of opinion should serve to initiate a dialog between listener (reader) and speaker in order to reach a common basis of understanding.

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Carotenoid Vitamin A Precursors and Analogs in Foods and Feeds

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A compilation has been prepared of carotenoid vitamin A precursors presently known in foods and feeds and their structure–vitamin activity relationship. Factors influencing their vitamin A value in the diet of various species, as well as factors influencing their content in natural and processed foods and feeds, are presented. The contribution of synthetically prepared carotenoids is acknowledged in extending nature's plan of coloring as *direct* additives to food products, or *indirectly* as additives to feeds in pigmenting poultry products.

Nature relies on a variety of compounds, namely carotenoids, chlorophylls, anthocyanins, and porphyrins, for the pigmentation of living matter. Carotenoids and/or carotenes are believed to have derived their name from the fact that they constitute the major pigment in the carrot root, *Daucus carota*. A wide variety of foods and feeds—yellow vegetables, tomatoes, apricots, oranges, egg yolk, chicken, butter, shrimp, lobsters, salmon, trout, yellow corn, etc.—owe their color principally to carotenoids, as do certain food color extracts from natural sources such as palm oil, paprika, annatto, and saffron. It has been estimated that nature produces about 100 million tons of carotenoid pigments per year (108). Their varied functions include provitamin A activity, absorbers of light energy, oxygen transporters, light yellow to dark red food colorings, and probably other functions unknown at this time. Complexed with protein, green and blue colorations are achieved. According to Burnett (28), the form in which carotenoids exist in living cells and an elucidation of the varied roles projected still requires a considerable research effort.

Carotenoids are aliphatic or aliphatic–alicyclic structures composed of 5-carbon isoprene groups, usually eight, so linked that the two methyl groups nearest the center of the molecule are in positions 1:6 and all other lateral methyl groups are in positions 1:5; a series of conjugated C–C double bonds constitute the chromophoric system (118).

Classifications of the carotenoids have taken several forms. Chemically, they may be divided into (a) the carotenes, made up of carbon and hydrogen only, and (b) the oxycarotenoids containing oxygen in addition to carbon and hydrogen, subdivisions of which would be (c) the epoxy, (d) the furanoxyl, (e) the hydroxy or xanthophylls (monols, diols, polyols), (f) the methoxy, (g) the keto, (h) esters, etc. A different classification system subdivides the carotenoids into (i) acyclic, (j) monocyclic, and (k) bicyclic derivatives. Functionally, they can be divided into (l) provitamins A or vitamin A precursors, (m) vitamin A precursors which also function as animal tissue pigmenters, (n) animal tissue pigmenters without vitamin A activity, and (o) compounds which do neither. A vast literature on the identification, isolation, chemistry, and properties of the carotenoids has been developed over the past 70 years (85, 104, 107, 108, 118, 191, 228, 229, 240).

CAROTENOID BIOSYNTHESIS

It has been concluded by Goodwin (85) that carotenoids are manufactured *de novo* only in the plant world. Biosynthetic investigations on plant and microbial preparations using radioactive tracers over the past several decades have established many of the reactions involved in carotenoid formation so that biogenetic pathways have been proposed. The enzyme systems involved are present in the cellular and/or subcellular components.

Acetate is considered the starting compound in the biosynthesis of the carotenoids. Two molecules of acetyl–CoA condense to form acetoacetyl–CoA which in turn condenses with acetyl–CoA again to produce β-hydroxy-β-methyl glutaryl–CoA, and through a reduction step forms mevalonic acid (MVA). MVA, in the presence of adenosine triphosphate (ATP), is converted to mevalonic acid phosphate and further phosphorylated to mevalonic pyrophosphate (MVAPP). In the presence of ATP and in combination decarboxylation and dehydration steps, MVAPP is converted to the important biological 5-carbon isoprene unit, isopentenyl pyrophosphate (IPP). IPP is first isomerized to dimethylallyl pyrophosphate (DMP) and then IPP and DMP condense to form geranyl pyrophosphate. This 10-carbon unit, by a continued condensation with IPP, yields farnesyl pyrophosphate and by one more condensation forms the 20-carbon unit, geranylgeranyl pyrophosphate, which in turn, by dimerization, forms phytoene, the basic 40-carbon acyclic carotenoid structure. In the cyclization of the acyclic carotenoids and the introduction of the oxygen moiety, there is more uncertainty about the pathways. The Goodwin group and the Chichester group favor the route of the zeacarotenes to the carotenes and oxycarotenoids, while the Porter group acknowledge that lycopene can be cyclized to the carotenes. A biosynthetic scheme for the carotenoids is shown as Figure 1. The routes of the biogenesis of a few of the bacterial pigments, such as rhodopin, etc., are also shown here. For a more detailed view of carotenoid biosynthesis in plants and microorganisms, recent reviews by Chichester (35, 36), Goodwin (82, 232), Jensen (112), and Porter (175) are available.

VITAMIN A ACTIVITY

The naturally synthesized pigments as components principally of plants are ingested by animals and metabolized by one or more pathways: (a) unselectively absorbed; (b) selectively absorbed; (c) chemically altered en route through the digestive tract or during the absorption process, prior to tissue storage or *in vivo* function. One transformation of practical significance to a variety of animal life, mammals, birds, amphibia and fish is the conversion of special structure carotenoids into vitamin A. Vitamin A is exclusively an animal compound present in practically all animal species.

In the USA, the average diet of the usually available foods is estimated to provide about 7500 IU of vitamin A per day, about 3500 IU derived from vegetables and fruits, 2000 IU

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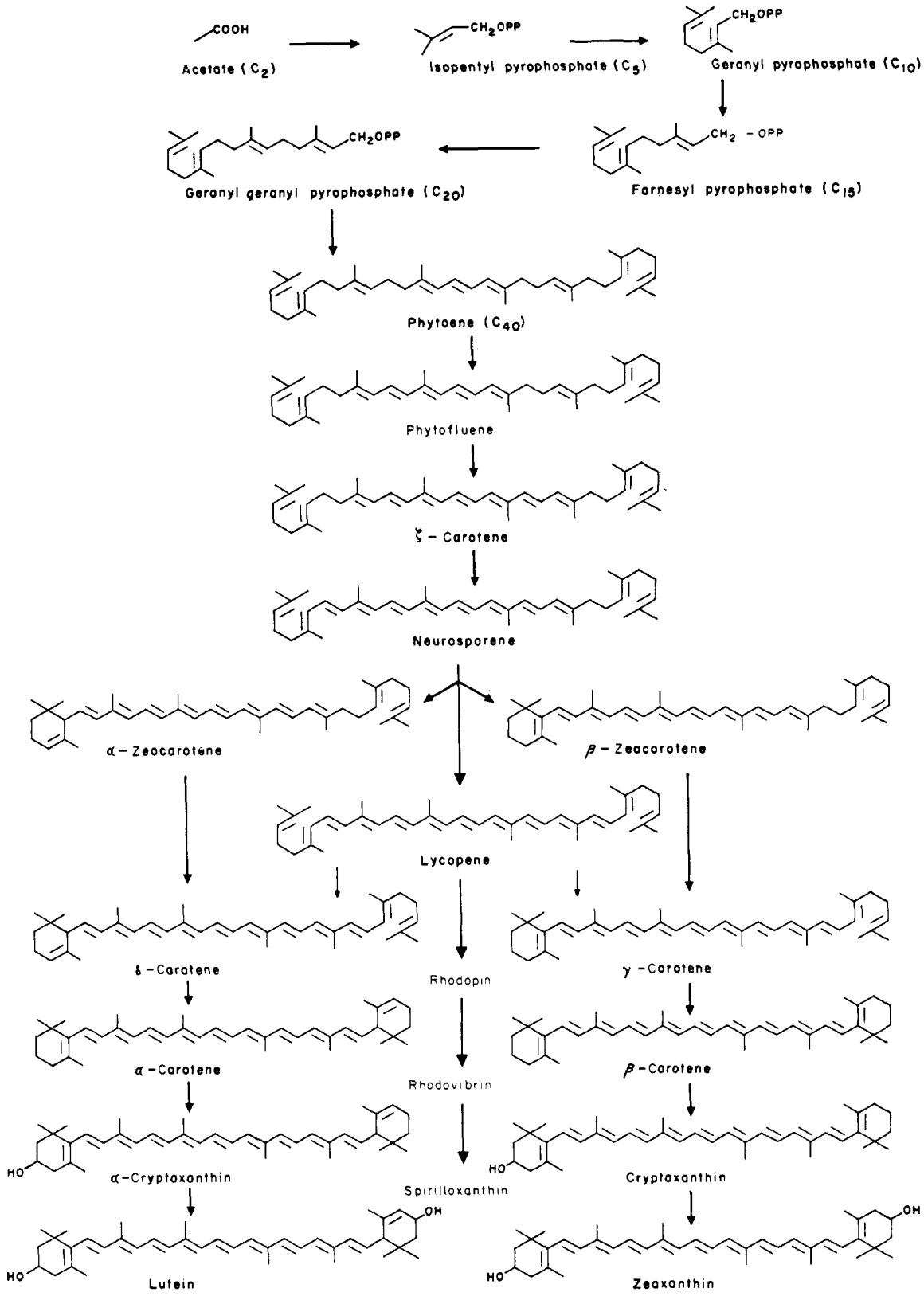

Figure 1. Biosynthetic scheme for some carotenoids

from fats, oils, and dairy products, and 2000 IU from meat, fish, and eggs. About 50% of the apparent USA vitamin A intake is in the form of provitamin A (182, 218). In a recent dietary survey (236) of adult diets, while there was a wide variation in daily supplies of both preformed vitamin A and carotenes, 60% exceeded the recommended daily allowance of 5000 IU. In Britain, the contribution (218) of dietary vitamin A needs from provitamin A is a third to nearly a half from fruits and vegetables. In India attempts are being made to increase consumption of leafy vegetables as one method of improving vitamin A intake. Thus, the carotenoid vitamin A precursors are an important source of vitamin A in the human diet.

Activity of the carotenoid vitamin A precursors can be illustrated in diverse manners: (a) by feeding the carotenoid to an intact animal under prescribed bioassay conditions and noting

Table I. Vitamin A Activity and Occurrence of Some Carotenoids

Carotenoid	Formula figure no.	Activity %	Reference	Occurrence	Reference
β-Carotene	1	100		Green plants, vegetables, carrots, sweet potatoes, squash, spinach, tomatoes, green peppers, pineapples, paprika, oranges, cranberries, figs, grapes, berries, apricots, peaches, prunes, apples, pears, strawberries, watermelons, wheat, corn, pasta products, palm oil, sorghum, hay, silage, algae, lichens, crustaceans, bivalves, eggs, fish, trout, alfalfa, tagetes meal	2, 4, 13, 21, 42, 43, 44, 49, 51, 54, 55, 59, 68, 76, 85, 96, 99, 102, 112, 124, 126, 130, 144, 153, 158, 159, 169, 176, 189, 190, 191, 193, 180, 181, 196, 209, 213, 215, 218, 221, 230, 242
α-Carotene	1	50–54	66, 71, 114, 137, 138	Green plants, carrots, squash, corn, green peppers, watermelons, potatoes, apples, peaches, oranges, cherries, figs, berries, grapes, bananas, pineapples, pasta, bleached paprika, tagetes meal, trout, palm oil, chestnuts	4, 42, 43, 44, 55, 59, 76, 94, 102, 85, 126, 153, 158, 159, 189, 190, 207, 221, 230, 242
3,4-Dehydro-β-carotene	2-1	75	27, 106		
3,4,3′,4′-Bisdehydro-β-carotene	2-2	38	27, 103, 106, 138		
2,2′-Dimethyl-β-carotene	2-3	50	69		
1,1′-Bisdemethyl-1,1′-bisethyl-β-carotene	2-4	41	207		
γ-Carotene	1	42–50	64, 87, 137, 233	Carrots, sweet potatoes, corn, tomatoes, algae, some fruits, apricots, watermelons, microorganisms, palm oil	7, 49, 54, 55, 124, 130, 158, 176, 180, 181, 193, 221
7′,8′-Dihydro-γ-carotene β-Zeacarotene	1	20–40	83, 172, 166	Corn, tomatoes, yeast, cherries	7, 76, 172, 181, 198
β-Carotene 5′,6′-monoepoxide	2-5	21	115, 116, 120, 166, 206	Plants, potatoes, red pepper	118, 171
α-Carotene 5,6-monoepoxide	2-6	25	116, 117, 119, 121	Plants, flowers, bleached paprika	59, 118, 121, 191
β-Carotene 5,6: 5′,6′-diepoxide	2-7	Active *ca.* 15	117, 120, 206	Plants	126
β-Carotene 5′,8′-monofuranoxide Mutatochrome, Citroxanthin, Flavacin	2-8	50	84, 115, 120	Orange peel, red peppers, tomatoes, sweet potatoes, cranberries, algae, bleached paprika	42, 59, 68, 112, 176
β-Carotene 5,6-monoepoxide 5′,8′-monofuranoxide Luteochrome	2-9	Active *ca.* 14	120		
β-Carotene 5,8: 5′,8′-difuranoxide Aurochrome	2-10	Active	120		
4-Keto-β-carotene 4-Oxo-β-carotene Echinenone, Aphanin, Myxoxanthin	3	44–50	61, 118, 140, 166	Algae, sea urchin, Daphniae, Hydra, red sponge, brine shrimp, crustaceans	58, 129, 130, 131, 212, 213
3-Keto-β-carotene 3-Oxo-β-carotene	2-11	52	78, 140		
3,4-Dehydro-4′-keto-β-carotene	2-12	Active	27		
β-Semicarotenone	2-13	Active	132, 133, 134	Citrus, *M. exotica*, *T. trifolia*	237
3,4-Diketo-β-carotene Euglenanone	2-14	Prob. active	83	Algae	83
3-Hydroxy-β-carotene	1	50–60	63, 65, 74, 88, 114, 136	Yellow corn, green peppers, lichens, persimmons, papayas, lemons, oranges, prunes, apples, apricots, peaches, strawberries, cranberries, pineapples, paprika, pasta, eggs, poultry, tagetes meal	4, 42, 43, 44, 46, 48, 49, 55, 59, 76, 112, 191, 124, 130, 153, 159, 171, 176, 190, 193, 221, 230
3-Hydroxy-β-carotene 5′,6′-monoepoxide Cryptoxanthin monoepoxide	2-15	Prob. active		Potatoes, oranges, lemons, prunes, flowers	19, 44, 46, 51, 54, 55, 177

Table I. (*Continued*)

Carotenoid	Formula figure no.	Activity %	Reference	Occurrence	Reference
4-Hydroxy-β-carotene Isocryptoxanthin	3	48	62	Brine shrimp	58
Isocryptoxanthin methyl ether	2-16	52	62		
3,4-Dehydro-3′-hydroxy-β-carotene Anhydrolutein, Deoxylutein	2-17	Active *ca.* 10	26, 27, 241	Alfalfa meal, acidulated soybean soapstock	25, 27, 241
5,6-Dihydroxy-β-carotene	2-18	Active	118, 132, 133, 135		
3-Hydroxy-4-keto-β-carotene Hydroxyechinenone	2-19	Prob. active		Algae	130, 112
β-Apo-2′-carotenal[a]	2-20	Active	118, 122	Citrus fruit	191, 212, 214
β-Apo-8′-carotenal	3	72	5, 79, 80, 122, 125, 149, 234, 235	Citrus fruit, green plants, animal tissue, alfalfa meal, grass	41, 46, 212, 214, 216, 234, 235
β-Apo-10′-carotenal[b]	2-21	Active	5, 79, 80	Citrus fruit, green plants, alfalfa meal	212, 214, 216, 235
β-Apo-12′-carotenal	3	120	5, 79	Alfalfa meal	212
β-Apo-8′-carotenoic acid	3	Active	Unpublished	Corn, animal tissue	7, 231
3′,4′-Dehydro-17′-oxo-γ-carotene[c] Oxotorulene	2-22	Active	138	Microorganisms, yeast	191
β-Apo-8′-carotenoic acid Ethyl ester	2-23	25, 78	23, 100		
Citranaxanthin[d]	2-24	44	100	Citrus, *S. citrangequat*	239
Torularhodin	2-25	Active <50	118, 123, 124, 156, 166	Yeast, microorganisms, fungi	123, 124, 191, 199
Cryptocapsin	2-26	Prob. active		Red peppers	43, 113, 191
Lycopene	2-27	Inactive		Tomatoes, carrots, green peppers, pink citrus fruit, apricots, watermelons, microorganisms	43, 49, 51, 68 158, 190
3,3′-Dihydroxy-β-carotene Zeaxanthin	1	Inactive		Spinach, paprika, yellow corn, green peppers, fruits, pasta, poultry, eggs, bivalves, brine shrimp, algae	43, 44, 48, 54, 55, 58, 59, 77, 99, 155, 197, 196, 221, 230
3,3′-Dihydroxy-α-carotene Lutein	1	Inactive		Green leaves, corn, potatoes, spinach, green peppers, carrots, tomatoes, fruits, apples, pears, apricots, peaches, prunes, oranges, strawberries, cranberries, figs, grapes, blackberries, pineapples, alfalfa meal, tagetes meal, trout, algae, grass, poultry, eggs, pasta	4, 13, 42, 43, 44, 49, 54, 55, 68, 76, 99, 117, 126, 153, 161, 189, 190, 193, 196, 218, 221, 230
3,3′-Dihydroxy-4,4′-diketo-β-carotene Astaxanthin	3	Inactive		Oranges, crustaceans, lobster, fish, algae, Daphniae, trout	34, 55, 65, 118, 212
4,4′-Diketo-β-carotene Canthaxanthin Aphanicin	3	Inactive		Mushroom, trout, Daphniae, Hydra, microorganisms, algae, crustaceans, brine shrimp	38, 54, 55, 58, 95, 129, 131, 188, 212, 213, 215
Capsanthin	2-28	Inactive		Red peppers, paprika	43, 45, 59, 189
Capsorubin	2-29	Inactive		Red peppers, paprika	43, 45, 59
Bixin	2-30	Inactive		Annatto seeds	191

[a] β-Apo-4′-carotenal (138) and β-apo-4′-carotenoic acid (neurosporaxanthin) also are potential provitamin A compounds existing in nature (1); β-apo-14′-carotenal is also active (79). [b] β-Apo-8′-carotenol is also biologically active. [c] Sintaxanthin (238), another methyl ketone, is probably also active. [d] Hydroxy-dihydro-γ-carotene (104), a microbial pigment, is probably also active.

tissue changes, blood, liver, etc., or by effect on growth; and (b) by using a purified or partially purified carotenoid cleavage enzyme in contact with the carotenoid under *in vitro* conditions and measuring retinal formulation. With the *in vitro* enzyme approach, α-carotene, β-carotene, bis-3,3′,4,4′-dehydro-β-carotene, 3′,4′-dehydro-17′-oxo-γ-carotene, and β-apo-10′-carotenol have shown activity (138).

For general vitamin A activity in mammals, it has been previously shown that a carotenoid provitamin A compound must have at least one unsubstituted β-ionine ring and a polyene side-chain attached. The other end of the molecule may vary in cyclic or acyclic structure and be lengthened but not shortened to less than an 11-carbon polyene fragment. β-Carotene possesses two β-ionone rings, one at either end of a

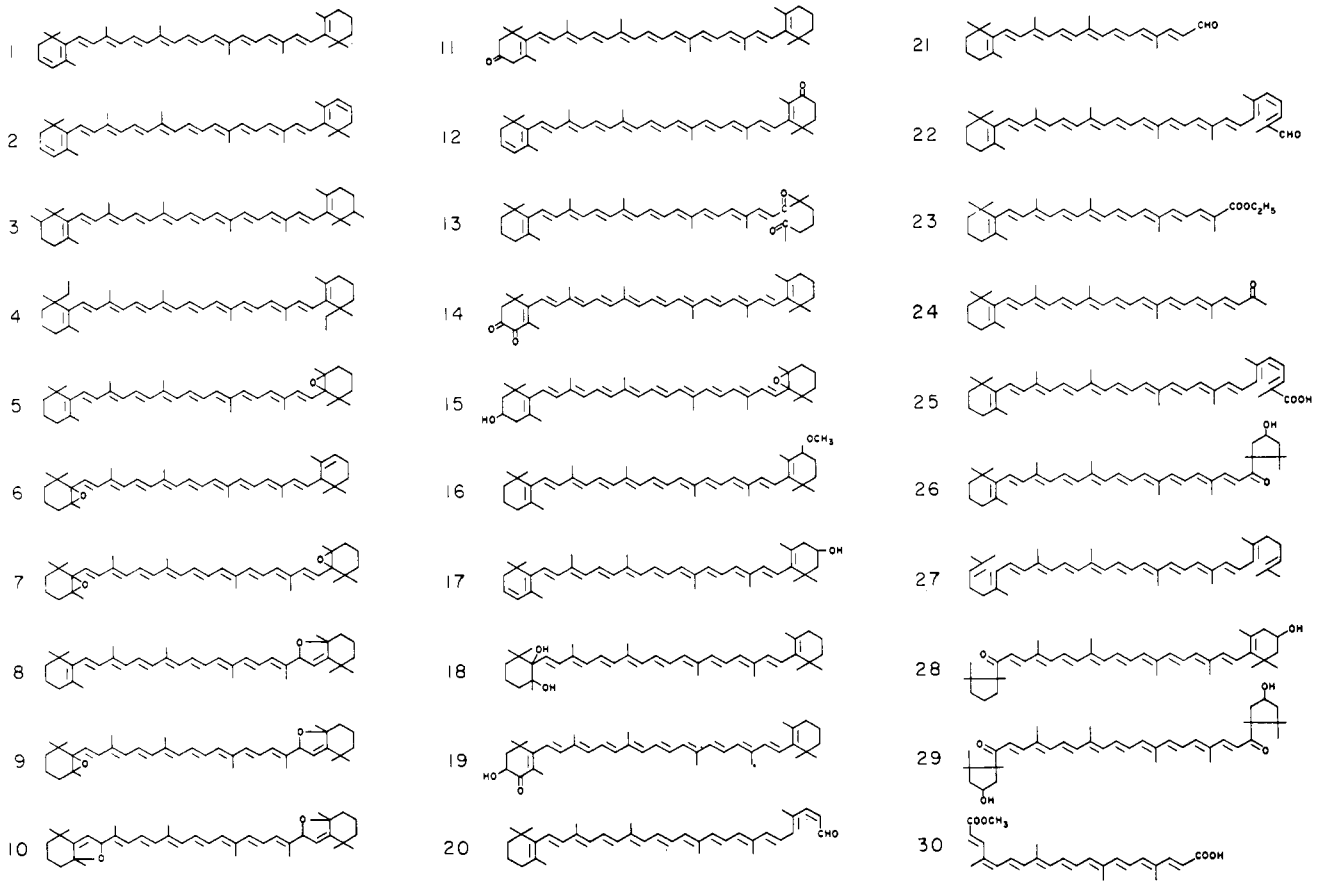

Figure 2. Structural formulas of carotenoids (refer to Table I)

long polyene chain, and is a provitamin A with high activity. Moore (157), over 40 years ago, unequivocally showed, by biological criteria, that β-carotene is converted to vitamin A, although Steenbock (201), over 50 years ago, first conceived that yellow color might be associated with biological activity. α- and γ-Carotenes are also biologically active at approximately half the β-carotene value. Tabulations of some of the carotenoids possessing vitamin A value and present in foods and feeds are shown in Table I and Figure 2. Activity figures presented are in terms of β-carotene as 100%.

Oxygen may be introduced into the molecule in certain locations and oxidation states. The β-apocarotenals are postulated to be degradation products, formed if terminal oxidation of the β-carotene molecule is the pathway for the conversion into vitamin A (80), and are biologically active when given orally (80, 125, 149) or by parenteral injection (154). The associated carotenals, carotenoic acids, and some acid esters are also active. The 5,6-monoepoxide, the 5,8-monofuranoxide, 5,6,5′,6′-diepoxide, and the 5,8,5′,8′-difuranoxide are believed to be vitamin A precursors despite the alteration of the β-ionone ring. It is assumed that *in vivo* these compounds are converted back to β-carotene. Other oxygen-containing carotenoids are biologically active, such as cryptoxanthin, isocryptoxanthin, 5,6-dihydroxy-β-carotene, citranaxanthin, and torularhodin, etc. Surmatis *et al.* (207) have reported that adding a methyl group to both rings of β-carotene in the 2,2′ positions does not destroy activity, nor does the replacement of methyl with an ethyl in the 1 or 1,1′ positions. However, the replacement of the methyl with an isobutyl group in the 1,1′ position does destroy biological activity. Oxidation that opens both rings of β-carotene destroys activity but, when only one ring is attacked, as in the case of β-semicarotenone, for example, activity remains. Dehydrogenation of the β-ionone ring, as in the case of 3,4-dehydro-β-carotene, still allows the compounds to be acitve. Budowski *et al.* (26) have shown that 3′-hydroxy-3,4-dehydro-β-carotene (anhydrolutein) acts as a precursor of vitamin A_2 in the chick and the mouse. This is the first instance of the formation of vitamin A_2 from a carotenoid by terrestrial animals. Vitamin A_2 is 3-dehydroretinol (2H less than A_1) and has about 40% of the activity of vitamin A. Activity is lost when hydroxyls and ketones are attached to both rings (positions 3 and 3′, 4 and 4′) or when both β-ionine rings are removed and the molecule becomes acyclic. Hydrogenation of both rings of β-carotene completely destroys its activity.

An exception to the above is the potential vitamin A value of astaxanthin and canthaxanthin for fish. Astaxanthin and canthaxanthin have been claimed to be provitamins for fish (86, 91) which, if true, would make these compounds important sources of vitamin A in the marine food chain. One recent report (32) claims that astaxanthin can replace vitamin A in the diet of the rat in the maintenance of the electroretinogram.

ACTIVITY OF ISOMERS

A great number of possible geometrical isomers can exist in the case of the carotenoids. With β-carotene, only 20 unhindered isomers are expected and 12 have been observed out of a theoretical 272 possibilities (240). The all-*trans*, the more stable geometrical form of the carotenoids, can undergo a *trans–cis* isomerization in solution; hence, it is sometimes difficult to decide whether a cis isomer occurs in nature or whether it is formed during its isolation because of the ease of *cis–trans* equilibrium mixtures to be formed under exposure to light, heat, and acids. Whether cis stereoisomers are active themselves or need to be first transformed in the tract to all-*trans* is not known. One view for the biological activity of

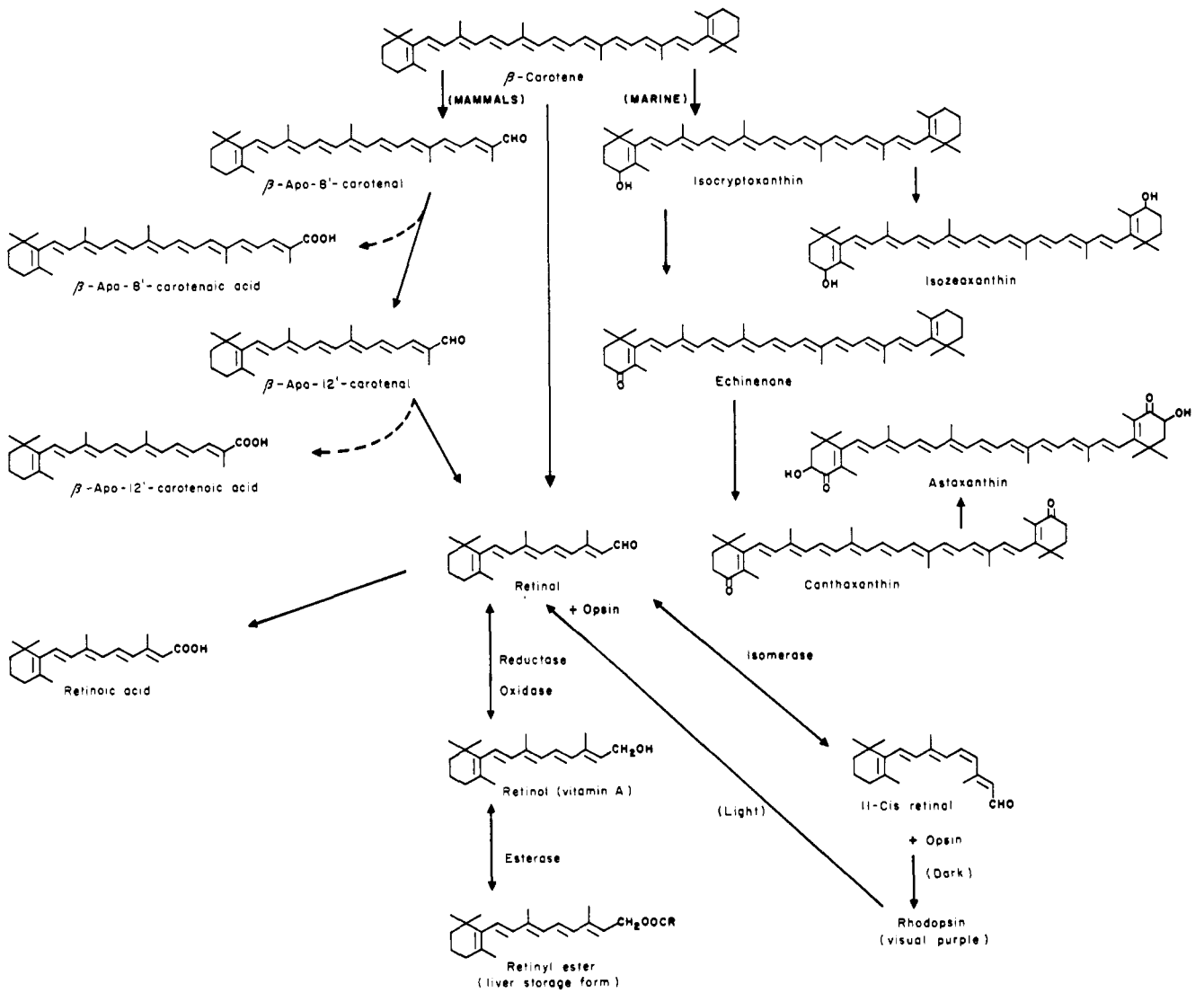

Figure 3. Metabolism schemes for β-carotene

the *cis*-carotene stereoisomers is that during digestion and/or absorption they are rotated, at least partially, on their asymmetric C atoms to the linear all-*trans* form.

The most complete study of the provitamin A activity of the stereoisomers of the carotenoids has been made by Zechmeister (240), in collaboration with Deuel (61). With the exception of pro-γ-carotene, cis isomers of the provitamin A structures have a lower biopotency than do the naturally occurring all-*trans* forms. The following provitamin A activities have been recorded for the stereoisomers tested on rats, all-*trans*-β-carotene being assigned a potency of 100%: 3- or 9-mono-*cis*-(neo-α-carotene U), 13%; 3,6- or 2,7-di-*cis*-(neo-α-carotene B), 16%; 3-mono-*cis*-(neo-β-carotene U), 38%; 3,6-di-*cis*(neo-β-carotene B), 53%; 15,15′-mono-*cis*-(β-carotene), 30–50%; 11,11′-di-*cis*-(β-carotene), 30%; 3-mono-*cis*-(neo-γ-carotene P), 19%; (mixed neo-γ-carotenes), 16%; poly or penta-*cis*-(pro-γ-carotene), 43%; 2-mono-*cis*-(neocryptoxanthin U), 27%; and the central mono-*cis*(neocryptoxanthin A), 42%. The provitamin activity of the procarotene is slightly higher in the chick than in the rat.

METABOLIC PATHWAY

Biosynthesis of vitamin A from β-carotene takes place mainly in the intestinal mucosa during absorption in an *in vivo* reaction sequence, namely (a) the cleavage of β-carotene in two molecules of retinal and (b) the reduction of retinal to retinol (81). Figure 3 is presented as a schematic pattern of metabolism. Conversion (79, 80, 81, 138) can be either by (a) symmetric or asymmetric fission or (b) terminal oxidation. The finding of the apocarotenals in biological and plant tissue supports the terminal oxidation approach. Furthermore, β-apo-12′-carotenal has a higher vitamin activity than β-carotene or β-apo-8′-carotenal. Yet, animals given large amounts of β-carotene do not yield chemically measurable amounts of the anticipated intermediates, presumably because they may exist as a fleeting stage, are protein bound, etc.

A carotenoid cleavage enzyme (15,15′-dioxygenase) has been demonstrated in a number of species of animals (81, 166). Other similar enzymes probably exist. In future evaluations, the enzyme approach may deserve higher priority in metabolism studies, considering the variables encountered in the intact animal bioassay and the need for greater volume of the compound in testing. On the other hand, (a) all compounds active in the intact animal, such as the β-carotene epoxides, do not respond to the *in vitro* test, and (b) the approach excludes terminal oxidation as an activity-producing mechanism.

In the marine food chain, β-carotene, in addition to being converted to vitamin A, forms oxycarotenoid structures. If adult brine shrimp, *Artemia salina*, are fed ^{14}C-β-carotene, a

Table II. Ratios of Biological Effectiveness of β-Carotene and Vitamin A for Various Animals (22)

Animal	Authority[a]	mg of β-carotene[b] equivalent to 1 mg of vitamin A alcohol	IU[c] preformed vitamin A per mg of β-carotene
Rat	International	2	1667
Mink	NAS-NRC	12	277
Fox	NAS-NRC	12	277
Dog	NAS-NRC	4 or 8	418-834
Poultry	NAS-NRC	2	1667
	NCAN	3	1112
	ARC	6	556
Horse	NAS-NRC	6-10	333-556
	NCAN	7	476
Dairy cattle	NAS-NRC	8-10	333-418
	ANRC	8	418
	NCAN	7	476
Beef cattle	NAS-NRC	8.3	400
	ANRC	8	418
	NCAN	7	476
Sheep	NAS-NRC	5.8-8.3	400-578
	ANRC	8	418
	NCAN	7	476
Swine	NAS-NRC	6.2	533
	NCAN	7	476
Man	MRC	6	556
	NAS-NRC	4	834
	BMA	6	556
	CCN	8	417
	NCAN	7	476

[a] ANRC, Animal Nutrition Research Council, N. America; ARC, Agricultural Research Council of Great Britain; BMA, British Medical Association; CCN, Canadian Council on Nutrition; MRC, Medical Research Council, Great Britain; NAS-NRC, National Academy of Sciences, National Research Council, United States; NCAN, National Committee on Animal Nutrition, Canada. [b] On the basis of all-*trans*-β-carotene. [c] 1 IU of vitamin A activity is usually regarded as ≈ to 0.6 μg of β-carotene or 0.3 μg of vitamin A alcohol or 0.344 μg of vitamin A acetate or 0.55 μg of vitamin A palmitate.

conversion takes place, first to echinenone and then to canthaxanthin (58, 131). Isocryptoxanthin and isozeaxanthin may be intermediates in some species.

RAT STUDIES

There are numerous reports that the vitamin A activity of β-carotene is dependent on the specific conditions of the rat assay. The addition of vitamin E (30, 96, 97, 129) at low levels in the growth assay enhances the vitamin A activity of β-carotene; larger quantities of vitamin E may be antagonistic to carotene utilization. The addition of ascorbic acid and other water-soluble vitamins (151), for example, cyancobalamin, the source of protein (101), the quantity and nature of the dietary fat (30), emulsifiers (61, 200), antioxidants (98), bile (165), and age are other variables which can exert a marked effect on the conversion and utilization of β-carotene by the rat. Administration of thyroxine or thyroid preparations (60) improves carotene absorption; thiouracil inhibits it. The evidence supports a β-carotene to vitamin A weight ratio of 2:1 (185) for low levels of β-carotene supplementation of the rat's diet. When carotene is fed at levels sufficient to elicit liver stores of vitamin A, the relative efficiency of conversion to and storage as vitamin A is markedly reduced (10, 111, 150, 156, 158). Data from different dietary levels from this laboratory (148) indicate that β-carotene provides from $^1/_3$ to $^1/_5$ the vitamin storage as equivalent unitage fed as vitamin A. This is an effective carotene to vitamin A ratio of 6:1 to 10:1 on a weight basis. From the foregoing illustration of the variable vitamin A performance of β-carotene in rats, it is not surprising that variable activity ratios have also been reported in other species.

POULTRY STUDIES

While true vitamin A has been fed as an ingredient of practical poultry feeds for decades, β-carotene is still present in many rations commonly fed to poultry. The vitamin A activity, as in the case of the rat, must be evaluated in relation to dietary dosage. At lower levels of feeding, where the criterion (33, 167) is growth, it is essentially equal to the activity found in rats, a β-carotene to vitamin A weight ratio of 2:1. Based on liver storage (33, 167) of vitamin A, where higher than minimum maintenance levels are fed, a marked reduction in efficiency of conversion takes place. Data from this laboratory (148) demonstrate that over a feeding range of 1000 to 5000 IU of vitamin A per pound of ration in the form of dry stabilized β-carotene or dry stabilized vitamin A palmitate, the vitamin A product consistently provides for 2.5 times the liver stores of vitamin A, as compared to the equivalent unitage from β-carotene, an effective weight basis ratio of 5 mg of β-carotene:1 mg of vitamin A.

LARGE ANIMAL STUDIES

Until recently, it has been normal agricultural practice to rely almost entirely on provitamin A to satisfy the requirement of adult cattle and horses, and sheep of all ages (173, 220). In a series of reports (92), Guilbert *et al.* reported that differences in the relative efficiencies of vitamin A and of carotene widen with increasing levels of dietary intake. At minimum levels adequate to prevent any detectable degree of nyctalopia for cattle, sheep, swine, and horses, the ratio of efficiency of vitamin A to carotene by weight is about 6.6:1, and at the minimum level that results in significant liver storage and successful reproduction is about 10:1. Using growth and reproduction in cattle, Guilbert and Loosli (93) found 6.6 times as much carotene on a weight basis to be required as vitamin A. Other studies (90) have also demonstrated a carotene to vitamin A equivalent efficacy ratio of 8 or 8:1 and higher. The relative potency of carotene for farm animals and the influencing factors such as dietary nitrates-nitrites have been reviewed by Beeson (12), Philips (173), Tiews (222), and McGillivary (155).

HUMAN STUDIES

Man absorbs and stores carotenoids relatively unselectively, and hence they can be found in liver, blood, and milk fat (81, 85). Adult fat is yellow, whereas that of the infant is white; hence, there is little placenta transfer (61). Information on feeding value of carotenoid vitamin precursors to humans is fragmentary due to the limited number of well-conducted trials (161, 197). A small recent study (164) showed the absorption of pure β-carotene to be better than that of β-carotene from foodstuffs. Murray and Campbell (161), recently reviewing this topic, summarize the existing data and point up the importance of digestibility and absorption aspects and the need for further research with man. They suggest, for the purpose of public health, recommendations that a provitamin A unitage three times that of vitamin A appears to be sufficiently accurate to use as a practical utilization ratio. This would be a weight ratio of 6 mg of β-carotene to 1 mg of vitamin A (alcohol). Other ratios (Table II) have been developed by other groups.

FACTORS INFLUENCING ACTIVITY

Some factors affecting biological activity of the carotenoid vitamin A precursors are: physical form of the carotenoid; its solubility; the state of isomerization; the dietary level of the carotenoid administered; the dietary fat level, its degree of unsaturation, and the state of oxidation; the presence of dietary antioxidants (tocopherol, ascorbic acid), and prooxidants (nitrites); the presence of dietary absorption inhibitors (mineral oil); the presence of adequate bile in the tract or added dietary emulsifiers; the type and adequacy of dietary protein; the role of thyroid-active compounds; the surface area of the digestive tract and the enzymic digestion efficiency; and the presence of disease and/or parasites in the organism. This subject is reviewed in detail elsewhere (22, 61, 162, 218, 219). The low solubility of carotenoids is recognized. It is difficult to make a stable solution of β-carotene in vegetable oil much greater than 0.05–0.1% (10, 157).

The type and adequacy of dietary protein is regarded to affect the biological value of carotene (183). The recent study of Pops *et al.* (174) of carotene metabolism in patients under starvation and malnutrition shows high serum carotene values, suggestive of poor conversion. However, this did not seem to be true in preschool children with mild protein–calorie malnutrition in another study (139).

It is apparent that β-carotene is best utilized at quite low levels in the ration (22, 185). A fairly well developed mechanism, in the absence of deterrents, exists to convert limited dietary intakes of β-carotene to the necessary vitamin A. As the ingested amount is increased, however, the efficiency of conversion decreases markedly. This phenomenon has been noted with both crystalline natural and synthetic all-*trans*-β-carotene, as well as stabilized dry β-carotene forms of synthetic origin (10). There is no single conversion ratio (222) for the transformation of β-carotene into vitamin A. Rather, it depends on the conditions of use. Ratios of biological effectiveness of β-carotene to vitamin A for various species, as recommended by different authorities in the USA, Canada, and Britain, are summarized in Table II (22). Rather than the theoretical ratio of 2:1, ratios of 4:1 to 8:1 are proposed.

OCCURRENCE

Naturally occurring carotenoids, for which the structures of well over 100 are known (1, 113), plus the modifications which are man-made, now total several hundred compounds. How and in which chemical and physical form carotenoids occur in nature is of importance, particularly to the food technologist involved in the processing and development of new food products. Large amounts of carotenoids are present in nature in a very fine colloidal dispersion. The excellent stability of the natural water-dispersible carotenoids or protein or lipoprotein complexes of them is probably due to their ultrastructure, but the nature of these linkages which extend the coloration range of the carotenoids, in addition to stability, is little understood.

In higher plants, carotenoids are present in the chromoplasts (83, 85). In senescent plant tissue such as autumn leaves, the xanthophylls liberated from the disintegrating chloroplasts are esterified and thereby become more lipophilic. Fruit carotenoids are attached to proteins, as is obvious from the general properties of fruit juices. The coloring pigments are not uniformly distributed throughout the cells of the citrus fruit, for example, but are concentrated in minute structures, plastids or chromatophores, characteristic of the species from which they are obtained. Some are found in the cell wall and the cell sap or fluid. In the few roots (85) that contain significant amounts of carotenoids, carotenes in carrots, for example, are also located in the plastids in lipophilic droplets or globuli as filaments and crystals.

In human and animal tissues, carotenoids may be present in fats in dissolved form. Correlations between the β-carotene content of blood serum (in man and various animals) and particular albumin fractions separable by electrophoresis have been found by various authors, and these indicate some linkage between carotenes and proteins (61, 184).

A vast number of scientific papers concerning the distribution and varieties of carotenoids in foodstuffs and extensive monographs in this field exist (82, 83). The overall carotenoid pattern may vary from relatively simple mixtures to extremely complex ones. The simplest mixtures may be found in foods of animal origin, due to the limited ability of the animal to absorb, to modify, and to deposit carotenoids. The other extreme is the formidable array of carotenoids encountered in citrus products, dehydrated alfalfa meal, or paprika, for example, which are only recently succumbing to modern sophisticated analytical chemistry.

In ripening fruit the decrease in chlorophylls is frequently accompanied by an increase in concentration of carotenoids and in the ratio of carotenes to oxycarotenoids. Carotenoids in fruits, juices, and concentrates were reviewed by Bauernfeind (9) in 1958 and tabulations were presented on their distribution. The total carotenoid content (21) of fruits varies as will be noted in the following (mg/kg) figures: apples, 0.9–5.4; apricots, 35; black figs, 8.5; blackberries, 5.9; blueberries, 2.7; cherries, 5–11; cling peaches, 27; cranberries, 5.8; grapes, 1.8; Japanese persimmons, 54; lemons, 2.4; Navel orange pulp, 23; pears, 0.3–1.2; pomegranates, 0.2; strawberries, 0.6–1.5; tangerine pulp, 27; and tomatoes, 51. The occurrence of some of the individual carotenoids is indicated in Table I.

α- and γ-Carotenes occur more frequently in fruit than leaves. β-Carotene is found quite widely distributed in fruit, in a range of 1 to 60% of the total carotenoid content (2, 21). Lycopene is particularly noticeable in the tomato, pink grapefruit, pink orange, watermelon, and also some varieties of apricots. Fruit oxycarotenoids often are in an ester form and are frequently unique structures. Intensive investigations of the type of carotenoids (41, 54, 55) of the orange have revealed that over 50% are oxycarotenoid esters, about 25% are unesterified oxycarotenoids, and about 10% are carotenes.

β-Apo-8′-carotenal was isolated from orange and tangerine peel by Curl (41), from orange peels by Winterstein *et al.* (235), and from peel and pulp (together with β-apo-2′- and 10′-carotenal) by Thommen (212, 214). Curl (41) also reported 3-hydroxy-β-apo-8′-carotenal (β-citraurin) in orange peel. Tangerines also exhibit a complex spectrum of carotenoids (53) and generally have a higher color intensity than the orange. Lycopene and β-carotene account for 80% or more of the color of the pulp of Ruby Red Grapefruit (51). In citrus relatives, the deeply pigmenting ketones, semi-β-carotenone (237) and citranaxanthin (239) have been reported.

Color is an important characteristic of citrus juices because it is one of the criteria of present commercial standards. The total color is affected by location, variety, maturity, processing methods, etc. (45, 143). Frozen, six-fold concentrated Valencia orange juice contains about 40 mg/kg total carotenoids and the distribution of the carotenoids is similar to single strength juice (52).

Apricots are an excellent source of β-carotene. Curl (49) reported the major carotenoid distribution to be about 60% β-carotene, 5% lycopene, 5% γ-carotene, 4% cryptoxanthin, and 2% lutein. In contrast (144), β-carotene is about 10% of the total carotenoids of peaches. McCarty and Lesley (153) found peaches to contain eight pigments, five of which were identified as α-carotene, β-carotene, cryptoxanthin, lutein, and zeaxanthin. Curl (50) found cling peaches to contain about 30 carotenoids. Varietal differences are great (204). The carotenes of apricots and peaches (2) are quite stable during processing and storage (80%). Italian prunes (44) contain α,β,ζ-carotene, cryptoxanthin, and a variety of other oxycarotenoids. The carotenoids of other fruits have also been studied, namely, apples (76), grapes (42), pears (76), figs (42), passion fruit (178), lemons (46, 223), persimmons (48), pineapples (159), papaya (85, 118), cherries (76), cranberries (42), cloudberries (84, 85), elderberries (84), strawberries (76), blueberries (42), and blackberries (42). Curl, as previously noted, pioneered the use of countercurrent distribution plus column chromatography to separate the complex carotenoid mixtures of fruits. Dozens of carotenoids have been identified. These methods have not been widely used in vegetables and animal food products to identify the carotenoid distribution pattern. One of the problems with the countercurrent distribution technique is that artifacts and isomers may be formed.

Fresh spinach leaves contain 40 mg/kg of β-carotene. Other oxycarotenoids, lutein, zeaxanthin, violaxanthin, and neoxanthin are also present (99), as is the pattern of most higher plant tissue. Yellow corn (maize) is widely used as food in fresh form, canned and frozen, and also processed in the form of corn flour, meal, and grits. White *et al.* (230) reviewed the carotenoids of yellow corn, *Zea mays* L. and mentions zeaxanthin, zeaxanthin isomers, lutein, cryptoxanthin, neo-cryptoxanthin, and α-, β- and γ-carotene and hydroxy-α-carotene. Quackenbush *et al.* (180) more recently separated 11 carotenoid fractions in five corn strains and compared the theoretical provitamin A activity with bioassay results. Sweet corn contains 0.9–1.5 mg/kg of β-carotene and 2–3 mg/kg cryptoxanthin (193, 221). Carotenes, β-carotene predominating, make up the minor portion of the total carotenoids and oxycarotenoids, zeaxanthin predominating the major portion. The total carotenoid content ranges from 10 to 55 mg/kg. A recently discovered carotenoid in corn is β-zeacarotene (172). It has provitamin A activity.

Lycopene is by far the predominant carotenoid of tomatoes; β-carotene, γ-carotene, and various oxycarotenoids, a minor fraction, make up the remainder (21, 47). Total carotenoid content is 20–60 mg/kg (dry basis). Zubeckis (243) reports commercial varieties of raw tomatoes to contain 2.3–7.4 mg/kg of carotene. In a sampling of tomato juice, a range of 3.8–5.5 mg/kg of carotene was noted. Large variations both between brands and between samples from the same manufacturer were observed.

Fresh color is a reliable indicator of the provitamin A value of sweet potatoes. Sweet potatoes and carrots maintain their carotene content during cool storage in root form (72). Furthermore, processed sweet potato flakes canned under nitrogen gas retained their carotene and ascorbic acid content for 2 years (176, 203). Sweet potatoes (176), in addition to significant quantities of β-carotene, also contain ζ-carotene, hydroxy-ζ-carotene, β-carotene furanoxide, γ-carotene, and *cis*-cryptoxanthin epoxide. α, β-, and ζ-carotene, hydroxycarotene, cryptoxanthin, lutein, zeaxanthin, violaxanthin, luteoxanthin, auroxanthin, neoxanthin, and neochrome have been reported in green peppers by Curl (43). The carotenoid content of the white potato, squash, broccoli, peas, beans, spinach, and pumpkins has been reviewed also by Borenstein and Bunnell (21). Watermelon (158) contains α-carotene, β-carotene, ζ-carotene, γ-carotene, and lycopene. Garden varieties of carrots average 54 mg/kg total carotene with α-, β-, γ-, and ζ-carotene prevalent and lycopene present in some of the orange varieties (94). The oxycarotenoid fraction is usually small (21) and yellow varieties contain more oxycarotenoids and less carotenes (20). Carrots can be successfully frozen and canned or dried as a food source of carotene (60, 203, 227).

In nature, the carotenoids exist primarily in their all-*trans* form; however, cis forms do exist and may even be abundant, as in the case of pro-γ-carotene (21). According to Sweeney and Marsh (210), the principal *cis*-β isomer in broccoli is neo-β-carotene U, a mono-*cis* isomer. Cooking of broccoli (25 min) caused increases in neo-β-carotene U (24%) but not neo-β-carotene B (5%), a di-*cis* isomer. Similar results were obtained with all green vegetables studied, including brussel sprouts, spinach, collards, kale, beet greens, and endive. Carotene in sweet potatoes is chiefly all-*trans*-β-carotene. Cooking (40 min) produced large amounts of neo-β-carotene B (24%) and small amounts of neo-β-carotene U (5%). Similar results were obtained with carrots, red peppers, pumpkins, and squash. It seems obvious that the principal β-carotene isomer formed during the cooking of green vegetables is neo-β-carotene U, while that formed during cooking of red or yellow vegetables is neo-β-carotene B. α-Carotene, when present, performed in the same fashion as did β-carotene. The reason for the difference between the green and nongreen vegetables in carotene isomer formation is not known.

Of the vegetable oils that are widely consumed, palm oil has by far the highest concentration of carotenoids, usually 500 mg/kg or more occurring in the crude, unbleached oil. α- and β-Carotene are the major carotenoids present (102), usually in a 3:2 ratio. In Africa, palm oil can be a significant source of provitamin A (218). Carotenes predominate (0.5–6.0 mg/kg) in the butterfat compared to oxycarotenoids (less than 1 mg/kg). Egg yolks contain 3–90 mg/kg of carotenoids (21, 85). The primary pigments are lutein, zeaxanthin, and cryptoxanthin with some β-carotene and other carotenoids, depending on the diet of the hen.

Thommen and Gloor (215) reported recently that the sea trout, *Salmo trutta*, contained considerable quantities of canthaxanthin as well as astaxanthin and β-carotene. Astaxanthin and β-carotene have been found in salmon (83, 84). The major storage sites of the carotenoids in fish are the skin, muscle, and ovaries.

For large farm animals, green grass, immature and early bloom legumes, and grasses cut and quickly dried into hay provide the major sources of provitamin A (40). The most common carotene in all green leaves is β-carotene. Recently the β-apocarotenals have been observed in grass and alfalfa (216). More than 40 different carotenoids are present in dehydrated alfalfa meal (15). Best known of the seed products are yellow corn and corn gluten meal, which contain β-carotene, cryptoxanthin, some α-carotene, γ-carotene, and carotene isomers (16). In a chromatographic study by Moster *et al.* (160) of the carotenoids of a corn seedling, 18 individual structures were cited. The relative amounts vary with varieties and strains. Tagetes meal (4) contains α-cryptoxanthin, antheraxanthin, α-carotene, β-carotene, and lutein. Sorghums contain considerably less (209) than yellow

corn, but β-carotene can vary from a trace to 25% of the total pigment in yellow varieties.

FOOD COLOR EXTRACTS

Annatto includes a whole series of coloring preparations, all based on extracts of the tropical seed of the Annatto tree, *Bixa orellana*. The pigments are present in the thin resinous coating of the seeds, and the major component consists of bixin, a monomethyl ester of a dicarboxylic acid which, on saponification, yields the free dicarboxylic acid called norbixin. Oleoresin of paprika is the oil extract of paprika, *Capsicum annuum*. Capsanthin and capsorubin are generally accepted to be the main carotenoids in paprika. A study (59) recently carried out to investigate quantitatively the changes in the carotenoid pigments of paprika caused by the bleaching process demonstrated up to 54 pigments isolated from bleached and 37 unbleached paprikas; only 33 and 21, respectively, were identified. Saffron, a spice and yellow colorant, contains crocin, the digentiobioside of crocetin. The principal carotenoids in these extracts are not vitamin A precursors. Carrot extracts, carrot oil, and palm oil and extracts were originally available on the market and their main components were α- and β-carotene. However, many of these products disappeared after the advent of synthetic β-carotene in 1954.

FACTORS INFLUENCING CONTENT

Carotenoid and carotene content is influenced by the genetics and environment of the plant (17). In parasitic type plants, α structures predominate over β structures; carotenoid epoxides are favored in high altitudes (19). Special corn varieties with high carotenoid content can be bred (17, 24). A diurnal pattern exists in the carotene content of the leaf (217). Variation also exists in the leaves, from oldest to lowest. Interplant variation can be considerable. Maximal carotene and oxycarotenoid content occurs after germination, and in alfalfa (lucerne), clovers, and grasses reaches a maximum before flowering, then decreases (142). Plants at maturity can have 50% maximum carotenoid value or less. Drought reduces carotene content of plants. Destruction of carotenoids results from enzymic and photochemical processes in harvesting and exposure to weathering (18, 195). In alfalfa leaves, sunlight-sensitized destruction is 7–8% of the total pigment present, while enzymic destruction amounts to 27–28% (89). Livingston *et al.* (141) report oxycarotenoid losses from 28–73% and carotene losses from 0–33% during pilot and industrial scale alfalfa dehydration. The carotene content of carrots can be influenced unfavorably by herbicide application (187).

Carotenoid content is usually correlated with the green color of the hays and silage. The rate of decrease depends on temperature, amount of exposure to air and sunshine, original content, and other factors. Under average conditions, the carotene content of hay can be expected to decrease about 6–7% per month. When the ambient temperature rises, the rate of loss is significantly higher. The carotene content of natural feed ingredients shows a large variation between feeds and a large range of assay values for any particular forage or feed concentrate. Among the grain products common in ruminant nutrition, only yellow corn and its byproducts contain measurable amounts of carotene and cryptoxanthin. The individual and total carotenoid content, for example, of corn is not constant, nor is the ratio of the individual carotenoids (67), nor are the individual oxycarotenoids equally available (8).

Milling and processing of seeds for grains may destroy or remove a considerable portion of the carotenoid content (16). Flours, such as wheat, are frequently low (85). Furthermore, the bleaching stage in flour processing destroys the residual carotenes so that white bread may be produced. Where special yellow color is desired, such as in pasta products like macaroni, special wheats (durum) are grown and are carefully processed to produce the semolina and farina. The carotenoids are mainly responsible for the yellow color (56) and processing losses in pasta of 30 to 60% of the original color have been reported (190).

Factors influencing carotenoid production in ripening fruit are oxygen, light, and temperature (85). The bleaching, retorting, and freezing processes generally cause little loss of carotenoid content in vegetables and fruits. Heat, however, does isomerize the all-*trans* carotenoids to cis forms. Frozen foods and heat sterilized foods exhibit excellent carotenoid stability throughout their normal temperature shelf life with few exceptions. Purcell and Walter (177) have observed in dehydrated sweet potato flakes that the mechanism of carotenoid destruction differs from that observed in oxidative *in vitro* studies. In general, dehydrated and powdered fruits and vegetables have poor carotenoid stability unless carefully processed and promptly placed in a hermetically sealed inert atmosphere (203, 221) for storage.

ASSAY

Workers who have compared the biological response of a food or feed containing a mixture of carotenoids and their isomers point out that, while it is possible to work out a factor for each item to convert the chemical value to that obtained biologically, the biological evaluation is the only method which indicates the actual vitamin A activity of such a feed ingredient.

The chemical determination of carotene (and other vitamin A precursors) has been of particular interest since it is used as a faster alternate and less costly procedure to the bioassay as a measure of the provitamin A content of foods. Column chromatography has been the method most frequently used (57, 157). Bickoff (14) points out that most of the older chemical methods for β-carotene measured only total carotene, no allowance being made for the difference in activity of the various provitamin A carotenoids, α-, β-, and γ-carotenes and cryptoxanthin, etc., that might be present. The problem is further complicated by present knowledge that each of the carotenoids can exist in a number of stereoisomeric forms which have widely different biological properties. However, the widely used chromatographic procedure for determination of the β-carotene in feed ingredients such as the AOAC Method (6) fail to separate the isomers of lower biological potency from all-*trans*-β-carotene, and thus basically their biological contribution is overestimated. Results by these procedures are usually labeled "carotene," total carotene, or β-carotene in the literature. The various carotenoids as described can be separated by chromatographic procedures and the individual carotenoids determined spectrophotometrically. Quackenbush (179), on the basis of the results of a collaborative study, proposed that a pigment separation method replace the present AOAC method. In making a carotene determination, Freed (75) recommended that carrots be assayed for α-carotene and corn for cryptoxanthin. An assay procedure for the carotene isomers has been proposed by Sweeney and Marsh (210), and values are presented on some vegetables. Improved vitamin A values of some vegetables are also reported by Nageswara (163) using a column

Table III. Carotene Content and Vitamin A Value in Foods and Feeds

Food	Carotene, mg/kg *a*	Vitamin A equivalent, IU/g *b*	Vitamin A equivalent, IU/g *c*
Apples (raw)	0.3	0.5	0.9
Apricots (raw)	15	25	27
Apricot nectar (canned)			9.5
Apricots, dried (raw)	36	60	109
Apricots, dried (stewed)	12	20	
Avocados (raw)	1	1.7	2.9
Bananas (raw)	2	3.3	1.9
Blackberries (raw)	1	1.7	2
Cherries (raw)	1.2	2	1.1
Cherries, sour (canned)	5.0	8.3	6.6
Currants, black (raw)	2	3.3	2.3
Gooseberries (raw)	1.8	3	2.9
Melons, yellow (raw)	20	33	34
Mango, (raw)			48
Nectarines (raw)			16.5
Olives, green (canned)	1.5	2.5	3
Oranges and juice (raw)	0.5	0.8	2
Peaches (raw)	5	33	13.3
Peaches, dried (raw)	20	33	
Pears (raw)	0.1	0.2	0.2
Pineapples	0.6	1	0.7
Plums (raw)	2.2	3.7	3
Prunes, dried (raw)	10	17	21.7
Prunes, dried (stewed)	5	8.3	7.6
Raspberries (raw)	0.8	1.3	0.3
Rhubarb (raw)	0.6	1	0.8
Strawberries (raw)	0.3	0.5	0.6
Tangerines (raw)	1	1.7	4.2
Asparagus (boiled)	5	8.3	9
Beans, french (boiled)	5	8.3	5.4
Beans, runner (boiled)	3		
Beetgreens (boiled)	50	83	51
Broccoli (boiled)	25	42	25
Brussel sprouts (boiled)	4	6.7	5.2
Cabbage	3	5	1.3
Carrots (canned)	70	116	105
Cauliflower (boiled)	0.3	0.5	0.6
Celery (raw)	20	33	33
Chicory greens (raw)			40
Chives (raw)			58
Collards (boiled)			78
Corn, yellow (boiled)			4
Corn meal, yellow			5
Dandelion greens (boiled)			117
Endive (raw)	20	33	33
Kale (boiled)	50	83	83
Lettuce (raw)	10	17	3.3–19
Mint (raw)	110	183	
Mustard greens (raw)	50	83	58
Okra (boiled)			4.9
Parsley (raw)	80	133	85
Peas (boiled)	3	5	5.4
Peas, dried (boiled)	0.8	1.3	0.4
Spinach (boiled)	60	100	81
Squash, summer (boiled)			3.9
Squash, winter (boiled)			35
Sweet potatoes (boiled)			79
Tomatoes or juice (raw)	7	11.7	8–10
Tomatoes (canned)	5	8.3	9
Turnip greens (boiled)	60	100	63
Watercress (raw)	30	50	49

Feed	mg/kg *d*	IU/g *e*
Alfalfa, immature (fresh)	62	104
Alfalfa, early bloom (fresh)	44	73
Alfalfa (ensiled)	27	46
Alfalfa, wilted (ensiled)	19	31
Alfalfa, early bloom (hay)	115	191
Alfalfa, full bloom (hay)	32	54
Alfalfa, overripe (hay)	7	12
Alfalfa meal (15% protein)	102	107
Alfalfa meal (22% protein)	253	422
Bluegrass, immature (fresh)	117	195
Corn, yellow (dent)	4	7
Corn (ensiled)	12	20
Corn, mature (ensiled)	4	7
Orchard grass (hay)	30	49
Timothy, midbloom (hay)	47	79
Timothy, mature (hay)	5	8

[a] Carotene content from McCance and Widdowson (152). [b] Vitamin A values calculated from reference (152) on basis of 1 mg ≈ 1667 IU. [c] Vitamin A values from Watt and Merrill (226). [d] Carotene content from Crampton and Harris (40). [e] Vitamin A values from Crampton and Harris (40) on basis of 1 mg ≈ 1667 IU.

separation technique. Assays have also been developed for carotenoids added to food products (10, 170).

The "carotene" content of foods and feeds is summarized in Table III. Food and feed composition tables should show both carotene (3, 22, 93), expressed on a weight basis, as well as the vitamin A content. If expressed on an IU basis, the conversion factor used should be shown. Since most of the "carotene" values on foods in this tabulation were established in the early sixties and primarily before that time, they do not necessarily represent the true vitamin A value of present day food. At that period of time, knowledge was still incomplete on the types of provitamin A compounds, and the specific assays for them and their isomers were in an earlier stage of development. The recent studies on provitamin A content of vegetables of Sweeney and Marsh (210) and Nageswara (163) are most encouraging and these types of studies should be extended to current varieties of vegetables and fruits, so that eventually new food composition tables of vitamin A values can be established.

CHEMICAL SYNTHESIS

β-Carotene was first synthesized by three different groups (11) in 1950 and an industrial process reported by Isler *et al.* (107) enabled its production to be initiated about 1954. The synthesis of β-apo-8′-carotenal from C_{19} aldehyde was reported by Isler and coworkers in 1959 (186) and was developed into the first industrial preparation of this carotenoid. β-Apo-8′-carotenoic acid ethyl ester or ethyl β-apo-8′-carotenoate was also synthesized in 1959 (105) and is in commercial production. All three of these carotenoid vitamin A precursors have practical food and feed value (11) in that the first two are primarily employed directly as food colorants; β-apo-8′-carotenal also serves indirectly as an egg yolk pigmenter by its addition to the ration of the hen; β-apo-8′-carotenoic acid ethyl ester also serves indirectly as an egg yolk pigmenter and as an additive to growing poultry rations for pigmenting the skin of chickens used for broiling, frying, and roasting. Canthaxanthin, a carotenoid without vitamin A activity, is also synthesized on a commercial scale (110) and

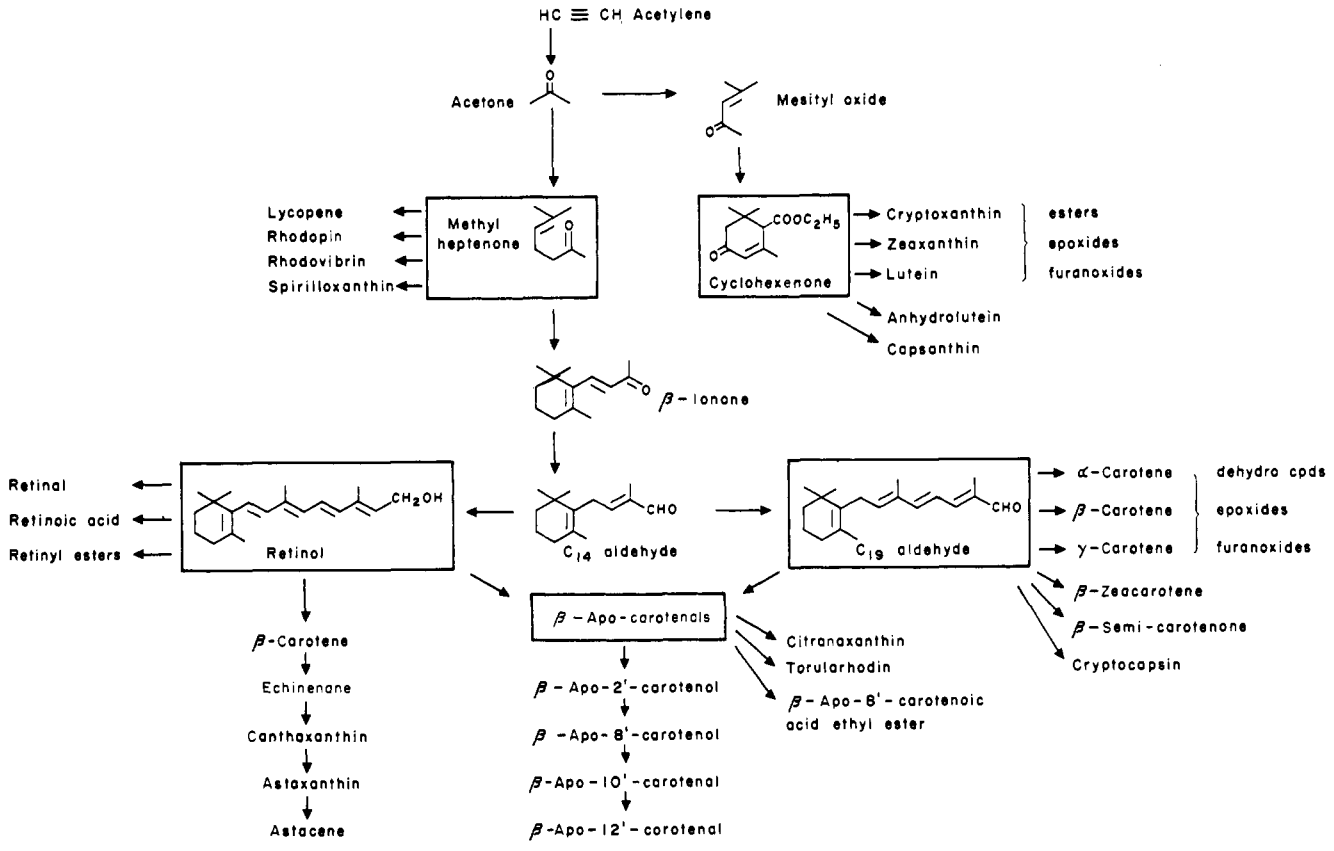

Figure 4. Chemical schemes of carotenoid syntheses

is useful as a direct food color and indirectly as a pigmenter adjuvant to be used with other oxycarotenoids.

Today, approximately 100 different carotenoids have been synthesized in the laboratory. Some of the routes of syntheses (107, 108, 109, 208) of many of the carotenoids discussed in this paper are shown in Figure 4. Solubility and stability difficulties encountered in handling crystalline carotenoids as food colors and feed pigmenters have been solved by the development of special forms. Two approaches have been primarily employed, namely (a) production of oil suspensions of micropulverized crystals and (b) development of emulsions or powder beadlet forms containing the carotenoid in supersaturated solution or in colloidal form (127, 162).

CAROTENOIDS AS PIGMENTERS

The term pigmenters is used to refer to carotenoids which, when present in or added to the animal ration, will color the body tissues such as skin and fat or animal products such as eggs, butter, and cheese. This indirect method of coloring food is as old as the animal kingdom.

β-Carotene, in addition to its role as a potent vitamin A precursor, is also a pigmenter for the dairy cow. Ingested β-carotene, not converted to vitamin A, is stored in the fatty deposits in the tissue and in the butterfat as well. Some α-carotene, cryptoxanthin, and lutein is also usually present. A great breed and species specificity exists in the metabolism of β-carotene (155). Ruminants like the Jersey and Guernsey dairy cow deposit carotene easily in their body fat depots and milk butterfat, while the Indian water buffalo, the goat, and the sheep have little color in the fat depots or milk butterfat. Since the dairy cow has a variable carotene intake over the year, as influenced by alternate feeding of fresh pasture and dry roughage and feed, the β-carotene and vitamin A content of the milk fluctuates accordingly (78, 195, 225).

Avian species convert ingested carotene to vitamin A (85) like mammals but differ from mammals in preferentially storing oxycarotenoids in liver, eggs, body fat, skin, feathers, and shanks. Pigmentation in the poultry field is concerned both with the skin color of meat birds (39) and the degree of yolk color. Moderately colored yolks are preferred for table use in many parts of the world; in certain other areas consumers want dark yolks (37). Highly colored yolks are desired for the commercial production of bakery products, noodles, mayonnaise, prepared cake mixes, and other egg products (31). The yellow-orange color of egg yolk is not synthesized by the hen, nor is the shank and skin color of the broiler; rather the color results from dietary transfer and deposit of selective oxycarotenoids present in the ration which the chicken consumes. Since properly pigmented poultry products are prized by consumers, the problem of pigmentation is primarily one of supplying a sufficient dietary level of the proper oxycarotenoids to accomplish the desired objective under defined conditions. Under natural conditions in the past, the chicken obtained the oxycarotenoids from grass and green plants. In modern practices with commercial poultry feeds, the pigmenting burden falls on yellow corn (maize), which does not always produce adequate results without the aid of significant quantities of alfalfa (lucerne) meal and/or corn gluten, etc.

Pigmentation studies with pure carotenoids have been run (29, 147, 162, 194, 202). Figure 5 shows the results of several of these trials wherein comparisons were made using the Roche Fan (224). In these studies (146, 147) a basal low pigment ration has been fed to laying hens. Other groups received either natural feedstuff ingredients containing a mixture of oxycarotenoids or the stabilized single synthetic carotenoid. In a laying ration for hens, composed essentially of white grain sources, or composed essentially of yellow corn, added β-apo-

Table IV. Stability of Carotenoids in Food Products

Product	Packaging	Added carotenoid	Method of addition	Carotenoid level: After processing	After storage 70–75° F (23° C): 2 mo	3 mo	4 mo	6 mo	12 mo
Apricot drink	Metal can	β-Carotene	Prior to canning	1.21 mg/6 fl oz		1.21		1.21(100)	1.16(96)
Apricot–orange drink	Metal can	β-Apo-8′-carotenal (beadlets)	Prior to canning	0.44 mg/6 fl oz		0.42		0.43(98)	
Butter	Paper, waxed carton	β-Carotene (emulsion)	At salting stage	4.7 mg/lb		4.2[b]		4.2[b](89)	
Butter	Paper, waxed carton	(beadlets)	At salting stage	5.2 mg/lb		4.4[b]		4.6[b](89)	
Butter	Paper, waxed carton	(gel)	At salting stage	7.9 mg/lb		7.2[b]		7.4[b](94)	
Butter	Paper, waxed carton	(gel)	In cream	7.8 mg/lb 3.23 mg/lb	3.29[b]	7.2[b]		7.3[b](94) 3.41[b](100)	
Butter	Paper, waxed carton	(gel)	In continuous processing	3.60 mg/lb				3.60[b](100)	
Cheese, spread, canned	Sealed can	β-Carotene (beadlets)	To the milk	1.7 mg/2 oz		1.8		1.7(100)	1.8(100) 1.7[e](100)
Cheese, cheddar	Waxed wedge	β-Carotene (beadlets)	To the milk	5.0 mg/lb		5.1[b]		5.4[b](100)	4.9[b](98)
Cheese, primary	Waxed wedge	β-Carotene (beadlets)	To the milk	5.4 mg/lb		5.6[b]			5.7[b,f](100)
		β-apo-8′-carotenal	To the milk	6.5 mg/lb		6.7[b]			7.8[b,f](100)
Cheese, primary	Waxed wedge	β-Carotene (A) and β-apo-8′-carotenal (B)	To the milk	A—2.4 mg/lb B—1.7 mg/lb A—4.3 mg/lb B—1.7 mg/lb					2.2[b](92) 1.6[b](99) 4.2[b](98) 1.6[b](94)
Cheese, processed	Film	β-Apo-8′-carotenal	To warm cheese	1.09 mg/lb	1.03[b]			1.06[b](97)	1.03[b](94)
Cheese, processed	Film	β-Carotene (A) and β-apo-8′-carotenal (B)	To warm cheese	A—2.1 mg/lb B—1.4 mg/lb A—3.9 mg/lb B—1.6 mg/lb					2.0[b](95) 1.3[b](93) 3.8[b](97) 1.4[b](88)
Cheese, processed	Paper and box	β-Apo-8′-carotenal (suspension)	To warm cheese	4.9 mg/lb		5.0[b]		5.1[b](100)	
Fruit juice drink	Metal can	β-Carotene (emulsion)	Prior to canning	1.32 mg/fl oz		1.38		1.32(100)	1.20(91)
Fruit juice drink	Metal can	β-Carotene	Prior to canning	0.96 mg/6 fl oz		0.92		0.87(91)	0.97(100)
Fruit juice base, chilled	Metal can	β-Carotene (emulsion)	Prior to canning	2.9 mg/8 fl oz		2.98[b]		2.86[b](96)	2.90[b](100)
Lard	Waxed paper carton	β-Carotene (suspension)	To warm fat	3.24 mg/lb				3.25(100)	
Margarine	Waxed paper carton	β-Carotene	To warm oil prior to churning	3.84 mg/lb	3.60			3.30(86) 3.66[b](95)	
Margarine	Waxed paper carton	β-Carotene	To warm oil prior to churning	3.51 mg/lb	3.48			3.30(94) 3.54[b](100)	
Margarine	Waxed paper carton	β-Carotene	To warm oil prior to churning	3.63 mg/lb	3.54			3.12(86) 3.42[b](94)	
Margarine	Waxed paper carton	β-Carotene	To warm oil prior to churning	3.33 mg/lb	3.06			3.12 (94) 3.14[b](100)	
Margarine	Waxed paper carton	β-Carotene	To warm oil prior to churning	3.90 mg/lb	3.54			3.24(83) 3.78[b](97)	
Orange beverage, carbonated	Glass bottle	β-Carotene (emulsion)	Prior to bottling	0.78 mg/6 fl oz				0.81(100)	0.66(85)
Orange beverage, carbonated	Glass bottle	β-Apo-8′-carotenal (alone)	Prior to bottling	3.5 mg/29 fl oz		2.73			
		with added ascorbic acid	Prior to bottling	3.70 mg/29 fl oz		3.31			
Orange beverage, carbonated	Glass bottle	β-Carotene (alone)	Prior to bottling	5.87 mg/29 fl oz		5.00			
		with added ascorbic acid		5.62 mg/29 fl oz		5.32			

Table IV. (*Continued*)

Product	Packaging	Added carotenoid	Method of addition	Carotenoid level: After processing	After storage 70–75° F (23° C): 2 mo	3 mo	4 mo	6 mo	12 mo
Orange drink, low calorie	Glass bottle	β-Carotene	Prior to canning	0.96 mg/10 fl oz	0.93	0.96		0.94(98)	0.92(96)
Orange drink	Metal can	β-Carotene and β-apo-8′-carotenal (3:1)	Prior to canning	2.75 mg/12 fl oz 2.71 mg/12 fl oz		2.71 2.68		2.64(97) 2.64(97)	
Orange drink	Enamel-lined can	β-Carotene (beadlets)	Prior to canning	1.73 mg/12 fl oz				1.67(97)	1.60[d](92)
Orange drink	Plain tin can	β-Carotene (beadlets)	Prior to canning	1.52 mg/12 fl oz				1.58(100)	1.60[e](92)
Orange drink	Enamel-lined can	β-Carotene (beadlets)	Prior to canning	0.56 mg/100 ml				0.51(91)	
Orange drink	Plain tin can	β-Carotene (beadlets)	Prior to canning	0.55 mg/100 ml				0.52(94)	
Orange juice, concentrate	Metal can	β-Carotene (beadlets)	Prior to canning	2.51 mg/16 fl oz		2.64		2.60(100)	2.88(100)
Orangeade base	Metal can	β-Carotene (beadlets)	Prior to canning	5.90 mg/6 fl oz		5.30		5.46(93)	
Pie filling	Metal can	β-Apo-8′-carotenal (beadlets)	Prior to canning	2.24 mg/kg		2.07		2.10(94)	2.00(89)
Pineapple–orange drink	Metal can	β-Carotene	Prior to canning	0.92 mg/6 fl oz		0.85		0.85(92)	
Popcorn oil	Pint bottles	β-Carotene (suspension)	To warm oil	13.6 mg/lb		12.7		13.1(96)	12.9(95)
Shortening	Sealed can	β-Carotene	To warm fat	3.80 mg/lb				3.71[b](98)	
Whole yolk, dry	Film	β-Carotene (beadlets)	In liquid yolk	5.5 mg/100 g	5.0		4.7	4.7(85)	
Whole yolk, dry	Film	β-Carotene (suspension)	In liquid yolk	7.7 mg/100 g	7.5		7.5	6.8(88)	
Whole yolk, dry	Film	β-Carotene (beadlets)	In liquid yolk	11.7 mg/100 g	10.4		9.4	9.5(81)	
Whole yolk, dry	Film	β-Carotene (emulsion)	In liquid yolk	14.1 mg/100 g	12.8		12.1	11.3(80)	
Whole yolk, frozen	Sealed containers	β-Carotene (beadlets)	In liquid yolk prior to freezing	16.9 mg/100 mg		16.5[a]			15.0[a](89)
Yolk product, dry	Film	β-Carotene	In liquid yolk	5.8 mg/100 g	5.8		5.4[c]		5.1(88)

[a] Storage temperature −5° F. [b] Storage temperature 40° F. [c] Storage temperature 86° F. Values in parentheses are percent retention during storage. [d] Storage period of 18 months. [e] Storage period of 21 months. [f] Storage period of 24 months.

8′-carotenoic acid ethyl ester or β-apo-8′-carotenal can provide the desired range of yolk color hue.

Broiler skin and shank pigmentation studies have also been made (145, 147), using a broiler ration of a white grain composition and little or natural oxycarotenoid present. Added β-apo-8′-carotenoic acid ethyl ester, like added natural carotenoid-bearing ingredients, can serve to give broilers the yellow skin color desired in the market place. Apocarotenal is not an efficient skin and shank pigmenter for broilers, therefore it is not recommended. Canthaxanthin will pigment both yolks and skin but must be used in combination with other oxycarotenoids (147). The choice of oxycarotenoid pigmenter or pigmenter combinations is governed by the desired objective, economics, and current availability of the products.

CAROTENOIDS AS FOOD COLORS

From the earliest recorded time, man has searched out the secret of the colors in nature to try to duplicate them and, hence, extend the plan of nature. The present availability of some of the synthetic carotenoids makes it possible to extend this plan to coloring processed and fabricated foods (21). In addition to its vitamin A value, carotene is probably the most widely added carotenoid for coloring foods. Employing the different market forms of β-carotene, it is technically and economically feasible to color fat or water-base foods such as butter, margarine, shortening, cheese, ice cream, macaroni products, breadings, frozen french fried potatoes, vegetable oils, salad dressing, whipped cream, cream toppings, coffee whiteners, popcorn oil, cocktail tidbits, dried or frozen yolks and whole eggs, eggnog, baked goods, cake mixes, candy, dietetic foods, puddings, creamed foods, soups, gelatin desserts, fruit juices, and beverages.

β-Apo-8′-carotenal, likewise, is used where an orange to reddish-orange shade is desired, such as in toppings, frostings, candies, confections, pastry fillings, cheese sauces, cheese spreads, cake mixes, soups, salad dressings, etc.

Canthaxanthin has unusually high tinctorial potency and is a useful food colorant in the red range. It has coloring value for tomato products such as soup, barbecue sauce, spaghetti sauce, and fruit drinks, salad dressings and simulated meat, and shrimp and lobster products. Stability in canned foods, baked goods, salad dressings, and processed cheese is good.

The use of β-apo-8′-carotenal in combination with β-carotene or either one in combination with canthaxanthin expands

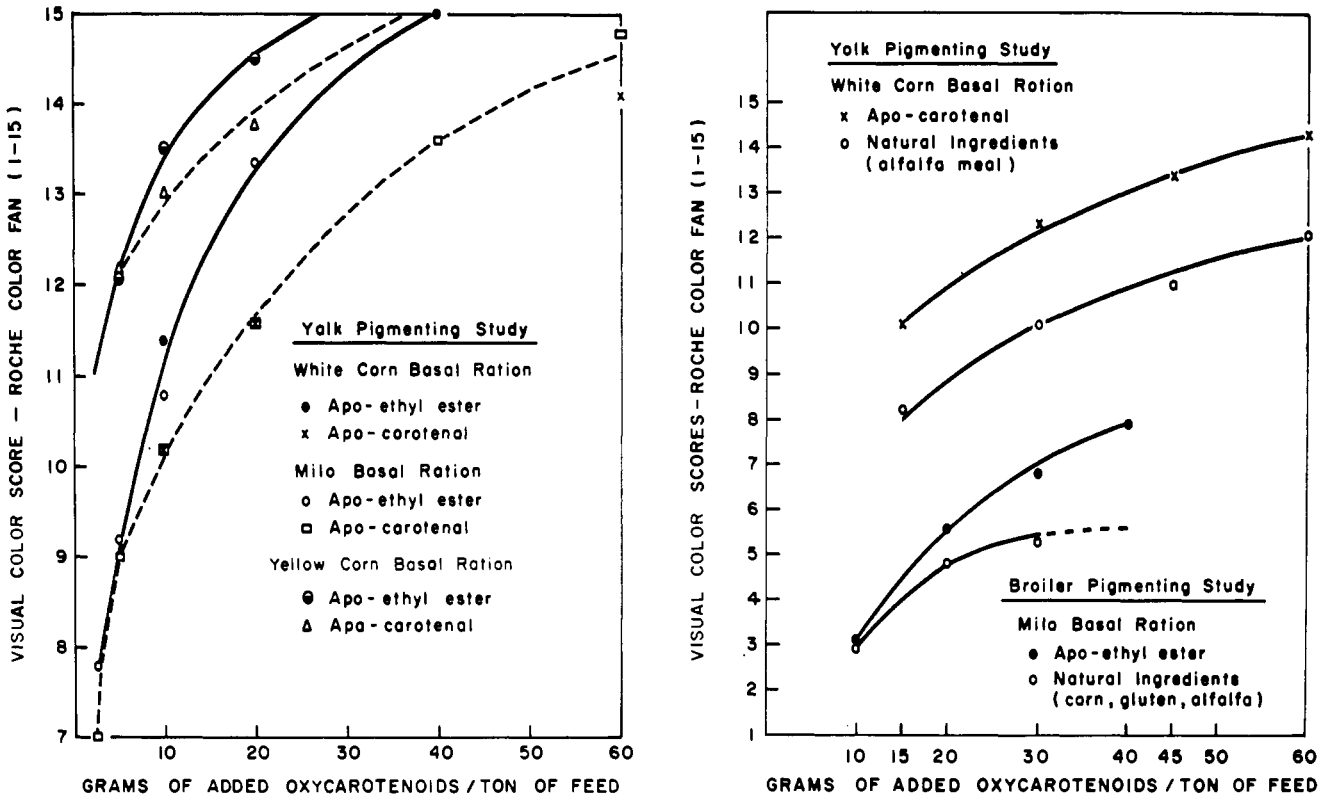

Figure 5. Performance of β-apo-8′-carotenal (146) and β-apo-8′-carotenoic acid ethyl ester (147) in pigmenting egg yolk and of β-apo-8′-carotenoic acid ethyl ester (145, 147) in pigmenting broilers as ingredients added to different types of rations compared to mixed oxycarotenoids in the form of natural ingredients

the color range of the individual compounds; for example, the use of β-apo-8′-carotenal and β-carotene in processed cheese or orange beverages. Ascorbic acid (21) has a stabilizing effect on β-carotene and β-apo-8′-carotenal in these beverages, contrasted to the instability of FD&C colors, which show fading in the presence of ascorbic acid under light exposure.

Table IV summarizes the results of small commercial or pilot-type trial runs on a number of food products containing added provitamin A type carotenoids as food colors. It must be recognized that these trials were made with the prevailing equipment and under the processing variables of that particular run and, hence, can only serve as a guide to expected performance under continuous standardized operating procedures. In general, however, added carotenoid vitamin A precursors are quite stable in food products when prepared under current food technological practices.

Vitamin A deficiency conditions resulting from inadequate diets are among the most common and serious, worldwide today (168, 183, 192). To be most effective, administered vitamin A and probably carotenoid vitamin A precursors as well should be given with an adequate protein intake for maximum vitamin A utilization (183). Hence, foods such as vitamin supplemented protein foods would be desirable carriers. In instances or at locations where it is desirable to improve the vitamin A content of the diet, the first choice of addition to food should be the economical forms of pure vitamin A esters which do not change the color of the end food product. Nevertheless, the use of a carotenoid vitamin A precursor such as β-carotene should not be overlooked, as it simultaneously provides both yellow color and vitamin value.

CAROTENOID COLOR STATUS

β-Carotene, β-apo-8′-carotenal, and canthaxanthin in the USA are accepted color additives for use in or on foods and are exempted from certification. The quantity of β-apo-8′-carotenal may not exceed 15 mg per lb of solid or semisolid food or per pint of liquid food. Canthaxanthin quantities may not exceed 30 mg per lb of solid or semisolid food or per pint of liquid food.

Based on the chronic toxicity data and specifications available, the Joint FAO/WHO Expert Committee on Food Additives (73) has classified β-carotene, β-apo-8′-carotenal, β-apo-8′-carotenoic acid ethyl ester, and canthaxanthin in class A, and they are therefore found acceptable for use in foods. Only three other coloring agents are in this class. Table V illustrates the worldwide approval of these four carotenoids as food colors.

SUMMARY

Over 100 different carotenoids exist in nature, many of which are components of foods and feeds regularly consumed by man and animals. Several dozen naturally occurring carotenoid vitamin A precursors are known and, *in toto*, they contribute significantly in supplying vitamin A value. Some, because of their rarity in distribution or their low concentration, are of minor significance. The more frequently recognized and the more valuable ones are probably the carotenes (α, β, γ), the epoxy-β-carotenes, the monohydroxy carotenes (cryptoxanthin), the monoketo-β-carotenes (echinenone), and the apocarotenals. The vitamin A activity of the naturally occurring carotenoids and compound structure–activity relationships are discussed. Factors influencing the utiliza-

Table V. Legal Status[a] of Carotenoids as Food Colors

Carotenoid	EEC[b] number	Countries generally permitting the respective carotenoid for the coloration of foodstuffs		
β-Carotene	E160 a	Argentina	Hungary	Portugal
		Australia	India	Rumania
		Austria	Iran	Spain
		Brazil	Israel	Sweden
		Canada	Japan	Switzerland
		Chile	Malaysia	Thailand
		Columbia	Mexico	Turkey
		Czechoslovakia	New Zealand	UK
		Denmark	Norway	USA
		EEC Countries	Panama	USSR
		East Germany	Paraguay	Union of S. Africa
		Finland	Peru	Uruguay
		Greece	Philippines	Venezuela
		Guatemala	Poland	Yugoslavia
β-Apo-8'-carotenal	E160 e	Argentina	Finland	Philippines
		Australia	Guatemala	Sweden
		Austria	India	Switzerland
		Canada	Malaysia	Thailand
		Chile	Mexico	UK
		Columbia	Norway	USA
		Czechoslovakia	Panama	Union of S. Africa
		Denmark	Paraguay	Uruguay
		EEC Countries	Peru	Venezuela
Canthaxanthin	E161 g	Argentina	Finland	Sweden
		Australia	Guatemala	Switzerland
		Austria	India	Thailand
		Canada	Malaysia	UK
		Chile	Mexico	USA
		Columbia	Norway	Union of S. Africa
		Czechoslovakia	Panama	Uruguay
		Denmark	Paraguay	Venezuela
		EEC Countries	Peru	
Ethyl ester of β-apocarotenoic acid (C_{30})	E160 f	Argentina	Finland	Switzerland
		Australia	Guatemala	Thailand
		Austria	India	UK
		Canada	Malaysia	Union of S. Africa
		Chile	Norway	Uruguay
		Columbia	Paraguay	Venezuela
		Czechoslovakia	Peru	
		Denmark	Sweden	
		EEC Countries		

[a] As of May 1970. [b] European Economic Community.

tion of β-carotene and presumably other carotenoid vitamin A precursors are presented. Currently accepted weight ratios of biological effectiveness of β-carotene and vitamin A for man and the various farm animals from various authoritative sources are 4:1 to 8:1 and higher, rather than the theoretical 2:1 ratio. The occurrence of the various carotenoid vitamin A precursors in foods and feeds is listed. Carotene content and vitamin A value summation tables on foods and feeds are available but some of the data are questionable in light of present knowledge. Biogenetic and metabolic pathways are briefly schematically diagrammed. The limitation of acrotenoid vitamin A precursor assay techniques and the need for a reassay of currently produced varieties and processed carotenoid vitamin A precursor-bearing foods is stressed. The contribution of pure carotenoid vitamin A precursors, produced by the chemical industry, and their practical value as *direct* food colorants to be added to processed and fabricated foods and their *indirect* role as ingredients of feeds as pigmenters, are indicated in extending nature's coloring scheme. There is growing universal acceptance of pure carotenoids as safe food colorants.

LITERATURE CITED

(1) Aasen, A. J., Jensen, S. L., *Acta Chem. Scand.* **19,** 1843 (1965).
(2) Aczel, A., *Konzerv Paprikaipar* 60 (1969).
(3) Adams, C. R., Bauernfeind, J. C., Proc. Minn. Nutr. Conf. Univ. Minn., Sept 8–10 (1963).
(4) Alam, A. U., Couch, J. R., Creger, C. R., *Can. J. Bot.* **46,** 1539 (1968).
(5) Al-Hasani, S., Parrish, D. B., *J. Nutr.* **94,** 402 (1968).
(6) AOAC, Methods of Analysis, 10th Ed., Ass. Offic. Anal. Chemists, Washington, D. C. (1965).
(7) Baraud, J., Benitez, R., Genevois, L., Maurice, A., *Compt. Rend.* **260,** 7045 (1965).
(8) Bartov, I., Bornstein, S., *Poultry Sci.* **36,** 796 (1967).
(9) Bauernfeind, J. C., Proc. Symp. Fruit Juice Concentrates, Bristol, England (1958).
(10) Bauernfeind, J. C., Osadca, M., Bunnell, R. H., *Food Technol.* **16,** 101 (1962).
(11) Bauernfeind, J. C., Rubin, S. H., Surmatis, J. D., Ofner, A., *Int. J. Vitamin Res.* **40,** 391 (1970).
(12) Beeson, W. M., *Fed. Proc.* **24,** 924 (1965).
(13) Benk, E., Dietl, R., Brixius, L., *Deut. Lebensm. Rundsch.* **63,** 110 (1967).
(14) Bickoff, E. M., "Determination of Carotene," *Methods of Biochemical Analysis*, Vol. 4, Interscience, New York, N.Y., 1957.
(15) Bickoff, E. M., Livingston, A. L., Bailey, G. F., Thompson, C. R., J. AGR. FOOD CHEM. **2,** 563 (1954); *J.A.O.A.C.* **37,** 894 (1954).
(16) Blessin, C. W., Brecher, J. D., Dimler, R. J., *Cereal Chem.* **41,** 543 (1964).

(17) Blessin, C. W., Brecher, J. D., Dimler, R. J., Grogan, C. O., Campbell, C. M., *Cereal Chem.* **40,** 463 (1963); *Crop Sci.* **3,** 213 (1963).
(18) Blessin, C. W., Dimler, R. J., Webster, O. J., *Cereal Chem.* **39,** 389 (1962).
(19) Bodea, C., *Pure Appl. Chem.* **20,** 517 (1969).
(20) Booth, V. H., *J. Sci. Food Agr.* **2,** 350, 353 (1951).
(21) Borenstein, B., Bunnell, R. H., *Advan. Food Res.* **15,** 195 (1967).
(22) Branion, N. D., Emslie, A. R. G., *Can. Dep. Agr. Publ.* 1238 (1966).
(23) Brubacher, G., Gloor, U., Wiss, O., *Chimia* **14,** 19 (1960).
(24) Brunson, A. M., Quackenbush, F. W., *Crop Sci.* **2,** 344 (1962).
(25) Budowski, P., Ascarelli, I., Gross, J., Nir, I., Bondi, A., *J. Amer. Oil Chem. Soc.* **41,** 441 (1964).
(26) Budowski, P., Ascarelli, I., Gross, J., Nir, I., *Science* **142,** 969 (1963).
(27) Budowski, P., Gross, J., *Nature (London)* **206,** 1254 (1965).
(28) Burnett, J. H., Chemistry and Biochemistry of Plant Pigments, T. W. Goodwin, Academic Press, 1965, p 381.
(29) Bunnell, R. H., Bauernfeind, J. C., Proc. 11th World's Poultry Congress, Mexico City, Sept (1958).
(30) Burns, M. J., Hauge, S. M., Quackenbush, F. W., *Arch. Biochem.* **30,** 341 (1951).
(31) Carlson, C. W., Shinnick, F. L., Sneeler, M. A., Halverson, A. W., *S. Dak. Farm Home Res.* **12**(2), 20 (1961).
(32) Carricaburu, P., Tayebi, B., Chardenot, P., *C. R. Acad. Sci Ser. D* **270,** 1282 (1970).
(33) Castano, F. F., Boucher, R. V., Callenbach, E. W., *J. Nutr.* **45,** 131 (1951).
(34) Cheesman, D. F., Prebble, J., *Comp. Biochem. Physiol.* **17,** 929 (1966).
(35) Chichester, C. O., *Pure Appl. Chem.* **14,** 215 (1967).
(36) Chichester, C. O., Nakayama, T. O. M., Biogenesis of Natural Compounds, 2nd ed., Pergamon press, 1967, p 641.
(37) Coles, R., *Agricultural Merchant* **42,** 103 (1962).
(38) Conney, J. J., Marks, H. W., Jr., Smith, A. M., *J. Bacteriol.* **92,** 342 (1966).
(39) Courtney, H. V., Branson, R. E., Texas A & M Univ., Bull. 989 April (1962).
(40) Crampton, E. W., Harris, L. E., Applied Animal Nutrition, 2nd ed., Freeman, San Francisco, Calif., 1969.
(41) Curl, A. L., *J. Food Sci.* **30,** 13 (1965).
(42) Curl, A. L., *J. Food Sci.* **29,** 241 (1964).
(43) Curl, A. L., J. AGR. FOOD CHEM. **12,** 522 (1964).
(44) Curl, A. L., *J. Food Sci.* **28,** 623 (1963).
(45) Curl, A. L., J. AGR. FOOD CHEM. **10,** 504 (1962).
(46) Curl, A. L., *J. Food Sci.* **27,** 171 (1962).
(47) Curl, A. L., *J. Food Sci.* **26,** 106 (1961).
(48) Curl, A. L., *J. Food Res.* **25,** 670 (1960).
(49) Curl, A. L., *J. Food Res.* **25,** 190 (1960).
(50) Curl, A. L., *J. Food Res.* **24,** 413 (1959).
(51) Curl, A. L., Bailey, G. F., *J. Food Res.* **22,** 63 (1957).
(52) Curl, A. L., Bailey, G. F., *Food Technol.* **13,** 394 (1959).
(53) Curl, A. L., Bailey, G. F., J. AGR. FOOD CHEM. **5,** 605 (1957)
(54) Curl, A. L., Bailey, G. F., *J. Food Sci.* **26,** 442 (1961).
(55) Curl, A. L., Bailey, G. F., J. AGR. FOOD CHEM. **4,** 156, 159 (1956).
(56) Dahle, L., J. AGR. FOOD CHEM. **13,** 12 (1965).
(57) Davies, B. H., Chemistry and Biochemistry of Plant Pigments, T. W. Goodwin, Academic Press, 1965, p 489.
(58) Davies, B. H., Hsu, W., Chichester, C. O., *Comp. Biochem. Physiol.* **32,** 69 (1970); **33,** 601 (1970).
(59) De La Mar, R. R., Francis, F. J., *J. Food Sci.* **34,** 287 (1969).
(60) Dellamonica, E. S., McDowell, P. E., *Food Technol.* **19,** 1597 (1965).
(61) Deuel, H. J. Jr., The Lipids, Their Chemistry and Biochemistry, Vol. III, Interscience (1957).
(62) Deuel, H. J. Jr., Ganguly, J., Wallcave, L., Zechmeister, L., *Arch. Biochem. Biophys.* **47,** 237 (1953).
(63) Deuel, H. J., Jr., Greenberg, S. M., Straub, E., Fukui, T., Chatterjee, A., Zechmeister, L., *Arch. Biochem.* **23,** 239 (1949).
(64) Deuel, H. J., Jr., Johnston, C., Summer, E., Polgar, A., Schroeder, W. A., Zechmeister, L., *Arch. Biochem.* **5,** 365 (1944).
(65) Deuel, H. J., Jr., Meserve, E. R., Johnston, C. H., Polar, A., Zechmeister, L., *Arch. Biochem.* **7,** 447 (1945).
(66) Deuel, H. J., Jr., Summer, E., Johnston, C., Polgar, A., Zechmeister, L., *Arch. Biochem.* **6,** 157 (1945).
(67) Dua, P. N., Day, E. J., Hill, J. E., Grogan, C. O., J. AGR. FOOD CHEM. **15,** 324 (1967).
(68) Edwards, R. A., Reuter, F. H., *Food Technol. Aust.* **8,** 352 (1967).
(69) Eugster, C. H., Trivedi, A. H., Karrer, P., *Helv. Chem. Acta* **38,** 1359 (1955).
(70) Euler, H. V., Karrer, P., Solmssen, U., *Helv. Chim. Acta* **21,** 211 (1938).
(71) Euler, H. V., Karrer, P., Zubrys, P., *Helv. Chim. Acta* **17,** 24 (1934).
(72) Ezell, B. D., Wilcox, M. S., *Plant Physiol.* **27,** 81 (1952).
(73) FAO/WHO Expert Committee on Food Additives Rept. No. 10, Oct (1967).
(74) Fraps, G. S., Kemmerer, A. R., *Ind. Eng. Chem. Anal. Ed.* **13,** 806 (1941).
(75) Freed, M., "Methods of Vitamin Assay," Interscience Publ. (1966).
(76) Galler, M., Mackinney, G., *J. Food Sci.* **30,** 393 (1965).
(77) Ganguly, J., Krinsky, N. I., Pinckard, J. H., Deuel, H. J., Jr., *Z. Physiol. Chem.* **295,** 61 (1953); *Arch. Biochem. Biophys.* **60,** 345 (1956).
(78) Gillam, A. E., Heilbron, I. M., Morton, R. A., Drummond, J. C., *Biochem. J.* **27,** 878 (1933).
(79) Glover, J., *Vitam. Horm. (New York)* **18,** 371 (1960).
(80) Glover, J., Redfearn, E. R., *Biochem. J.* **58,** 15P (1954).
(81) Goodman, D. S., *Amer. J. Clin. Nutr.* **22,** 963 (1969).
(82) Goodwin, T. W., Chemistry and Biochemistry of Plant Pigments, Academic Press (1965).
(83) Goodwin, T. W., *Wiss. Veroeff. Deut. Ges. Ernaehr.* **9,** 1 (1963); Biosynthesis of Vitamins and Related Compounds p 270 (1963).
(84) Goodwin, T. W., *Biochem. J.* **62,** 346 (1956).
(85) Goodwin, T. W., "Carotenoids, Their Comparative Biochemistry," Chem. Publ. (1954).
(86) Grangaud, R., Nicol, M., LeGall, J., Soussy, A., *Arch. Sci. Physiol.* **18,** 235 (1964).
(87) Greenberg, S. M., Calbert, C. E., Pinckard, J. H., Deuel, H. J., Jr., Zechmeister, L., *Arch. Biochem.* **24,** 31 (1949).
(88) Greenberg, S. M., Chatterjee, A., Calbert, C. E., Deuel, H. J., Jr., Zechmeister, L., *Arch. Biochem.* **25,** 61 (1950).
(89) Griffith, R. B., Thompson, C. R., *Botan. Gaz.* **111,** 165 (1945).
(90) Grifo, A. P., Jr., Rousseau, J. E., Jr., Eaton, H. D., Gosslee, D. G., *J. Dairy Sci.* **43,** 1006 (1960); *J. Anim. Sci.* **18,** 288 (1959).
(91) Gross, J., Budowski, P., *Biochem. J.* **101,** 747 (1966).
(92) Guilbert, H. R., Hart, G. H., *J. Nutr.* **19,** 91 (1940).
(93) Guilbert, H. R., Loosli, J. K., *J. Anim. Sci.* **10,** 22 (1951).
(94) Harper, R. H., Zscheile, F. P., *J. Food Res.* **10,** 84 (1945).
(95) Haxo, F., *Botan. Gaz.* **112,** 228 (1950).
(96) Hebert, J. W., Morgan, A. F., *J. Nutr.* **50,** 175 (1953).
(97) Hickman, K. C. D., Kaley, M. W., Harris, P. L., *J. Biol. Chem.* **152,** 303 (1944).
(98) High, E. G., *Arch. Biochem. Biophys.* **60,** 456 (1956).
(99) Hirayama, O., Oida, H., *J. Agr. Chem. Soc. Jap.* **43,** 423 (1969).
(100) Hoppe, P., 14th Proc. World's Poultry Congress (Madrid) pp 419 (1970).
(101) Hove, E. L., *Fed. Proc.* **11,** 446 (1952).
(102) Hunter, R. F., Krakenberger, R. M., *Biochem. J.* **40,** 492 (1946).
(103) Inhoffen, H. H., Raspe, G., *Ann. Chem.* **594,** 165 (1955).
(104) Isler, O., "Carotenoids," Birkhauser Verlag, Basle (1971).
(105) Isler, O., Guex, W., Rüegg, R., Ryser, G., Saucy, G., Schwieter, U., Walter, M., Winterstein, A., *Helv. Chim. Acta* **42,** 864 (1959).
(106) Isler, O., Lindlar, H., Montavon, M., Rüegg, R., Zeller, P., *Helv. Chim. Acta* **39,** 274 (1956).
(107) Isler, O., Lindlar, H., Montavon, M., Rüegg, R., Zeller, P., *Helv. Chim. Acta* **39,** 249 (1956).
(108) Isler, O., Rüegg, R., Schwieter, U., *Pure Appl. Chem.* **14,** 245 (1965).
(109) Isler, O., Rüegg, R., Schudel, P., *Chimia* **15,** 208 (1961).
(110) Isler, O., Schudel, P., *Wiss. Veroeff. Deut. Ges. Ernaehr.* **9,** 54 (1963).
(111) James, W. H., El Gindi, I. M., *J. Nutr.* **51,** 97 (1953).
(112) Jensen, S. L., *Pure Appl. Chem.* **20,** 421 (1969).
(113) Jensen, S. L., *Pure Appl. Chem.* **14,** 227 (1967).
(114) Johnson, R. M., Bauman, C. A., *Arch. Biochem.* **19,** 493 (1948); **22,** 122 (1949).
(115) Karrer, P., *Fortschr. Chem. Org. Naturst.* **5,** 1 (1948).
(116) Karrer, P., *Helv. Chim. Acta* **28,** 474, 1146 (1945).
(117) Karrer, P., *Doc. Ophthalmol.* 259 (1938).
(118) Karrer, P., Jucker, E., "Carotinoide," Elsevier Publ. (1950).
(119) Karrer, P., Jucker, E., *Helv. Chim. Acta* **28,** 471 (1945).
(120) Karrer, P., Jucker, E., *Helv. Chim. Acta* **28,** 427 (1945).
(121) Karrer, P., Jucker, E., Rutschmann, J., Steinlin, K., *Helv. Chim. Acta* **28,** 1146 (1945).
(122) Karrer, P., Rüegger, A., Geiger, A., *Helv. Chim. Acta* **21,** 1171 (1938).
(123) Karrer, P., Rutschmann, J., *Helv. Chim. Acta* **29,** 355 (1946).
(124) Karrer, P., Rutschmann, J., *Helv. Chim. Acta* **26,** 2109 (1943); **28,** 795 (1945).
(125) Karrer, P., Solmssen, U., *Helv. Chim. Acta* **20,** 682 (1937).
(126) Kasim, M., *Nahrung* **11,** 405 (1967).
(127) Klaui, H. M., Proc. Inst. Food Sci. and Tech. Special Color Sym UK. December (1968); *Wiss. Veroeff. Deut. Ges. Ernaehr.* **9,** 390 (1963).
(128) Koehn, C. J., *Arch. Biochem.* **17,** 337 (1948).
(129) Krinsky, N. I., *Comp. Biochem. Physiol.* **16,** 181 (1965).
(130) Krinsky, N. I., Goldsmith, T. H., *Arch. Biochem. Biophys.* **91,** 271 (1960).
(131) Krinsky, N. I., Lenhoff, H. M., *Comp. Biochem. Physiol.* **16,** 189 (1965).

(132) Kuhn, R., Brockmann, H., *Justus Ann. Liebigs Chem.* **516,** 95 (1935).
(133) Kuhn, R., Brockmann, H., *Ber.* **67,** 1408 (1934).
(134) Kuhn, R., Brockmann, H., *Ber.* **66,** 1319 (1933).
(135) Kuhn, R., Brockmann, H., *Ber.* **65,** 894 (1932).
(136) Kuhn, R., Grundmann, C., *Ber.* **67,** 593 (1934).
(137) Kuhn, R., Brockmann, H., Scheunert, A., Schieblich, M., *Z. Physiol. Chem.* **22**(1), 129 (1933).
(138) Lakshmanan, M. R., Pope, J. L., Olson, J. A., *Fed. Proc.* **28,** 490 (1969); *Biochem. Biophys. Res. Commun.* **33,** 347 (1968).
(139) Lala, V. R., Reddy, V., *Amer. J. Clin. Nutr.* **23,** 110 (1970).
(140) Lederer, E., Moore, T., *Nature (London)* **137,** 996 (1936).
(141) Livingston, A. L., Knowles, R. E., Nelson, J. W., Kohler, G. O., J. AGR. FOOD CHEM. **16,** 84 (1968).
(142) Livingston, A. L., Smith, D., Carnahan, H. L., Knowles, R. E., Nelson, J. W., Kohler, G., *J. Sci. Food Agr.* **19,** 632 (1968).
(143) MacKinney, G., The Orange: Biochemistry and Physiology, Univ. Calif. Publ. (1961).
(144) MacKinney, G., Arnoff, S., Borenstein, B. T., *Ind. Eng. Chem. Anal. Ed.* **14,** 391 (1942).
(145) Marusich, W. L., *Feedstuffs* **42**(10), 30 (1970).
(146) Marusich, W. L., *Feedstuffs* **39**(4), 48 (1967).
(147) Marusich, W. L., Bauernfeind, J. C., *Poultry Sci.* **49,** 1555, 1566 (1970).
(148) Marusich, W. L., Bauernfeind, J. C., *Poultry Sci.* **42,** 949 (1963).
(149) Marusich, W. L., De Ritter, E., Vreeland, J., Krukar, R. J., J. AGR. FOOD CHEM. **8,** 390 (1960).
(150) Matsumuro, H., Kitagawa, S., Kaga, A., *Ann. Rep. Natl. Inst. Nutr. (Tokyo)* 74 (1959).
(151) Mayfield, H. L., Roehm, R. R., *J. Nutr.* **58,** 203, 483 (1956).
(152) McCance, R. A., Widdowson, E. M., The Composition of Foods, Med. Res. Council Spec. Rept. Ser. 297 (1960).
(153) McCarty, C. D., Lesley, J. W., *Proc. Amer. Soc. Hort. Sci.* **64,** 289 (1954).
(154) McGillivray, W. A., *Brit. J. Nutr.* **15,** 313 (1961).
(155) McGillivray, W. A., World review of Nutrition and Dietetics 2, 133 Karger, Basle (1960).
(156) Moore, T., "Vitamin A," Elsevier Publ. (1957).
(157) Moore, T., *Biochemistry* **24,** 692 (1930).
(158) Morgan, R. C., *J. Food Sci.* **32,** 275 (1967).
(159) Morgan, R. C., *J. Food Sci.* **31,** 213 (1966).
(160) Moster, J. B., Quackenbush, F. W., Porter, J. W., *Arch. Biochem. Biophys.* **38,** 287 (1952).
(161) Murray, T. K., Campbell, J. A., *Can. Dep. Agr. Publ.* 1238 (1966).
(162) Nadai, J., Brubacher, G., *Int. Encycl. Food Nutr.* **9,** 449 (1970).
(163) Nageswara, R. C., *J. Nutr. Diet.* **4,** 10 (1967).
(164) Nageswara, R. C., Narasinga, R., *Amer. J. Clin. Nutr.* **23,** 105 (1970).
(165) Olson, J. A., *J. Biol. Chem.* **236,** 349 (1961).
(166) Olson, J. A., Lakshmanan, M. R., The Fat-Soluble Vitamins, Deluca, H. F., and Suttie, J. W., University of Wisconsin press, 1971, p. 213.
(167) Olsen, E. M., Harvey, J. D., Hill, D. C., Branion, H. D., *Poultry Sci.* **38,** 929 (1959).
(168) Oomen, H. A., McLaren, D. S., Escapini, H., *Trop. Geogr. Med.* **16,** 271 (1964).
(169) Orth, A., Koch, G., *Wiss. Veroeff. Deut. Ges. Ernaehr.* **9,** 363 (1963).
(170) Osadca, M., De Ritter, E., Bunnell, R. H., *J.A.O.A.C.* **49,** 1078 (1966).
(171) Pendlington, S., Dupont, M. S., Trussell, F. J., *Biochem. J.* **94,** 25 (1965).
(172) Petzold, E. N., Quackenbush, F. W., McQuistan, M., *Arch. Biochem. Biophys.* **82,** 117 (1959).
(173) Phillips, W. E. J., *Can. Dep. Agr. Publ.* 1238 (1966).
(174) Pops, M. A., Merrill, S., Schwabe, A. D., *Gastroenterology* **56,** 1190 (1969).
(175) Porter, J. W., *Pure Appl. Chem.* **20,** 449 (1969).
(176) Purcell, A. E., *Food Technol.* **16,** 99 (1962).
(177) Purcell, A. E., Walter, W. M., J. AGR. FOOD CHEM. **16,** 650 (1968).
(178) Pruthi, J. S., Lal, G., *J. Food Res.* **23,** 505 (1958).
(179) Quackenbush, F. W., *J.A.O.A.C.* **53,** 186 (1970).
(180) Quackenbush, F. W., Firsch, J. G., Rabourn, W. J., McQuistan, M., Petzold, E. N., Kargl, T. E., J. AGR. FOOD CHEM. **9,** 132 (1961).
(181) Raymundo, L. C., Griffiths, A. E., Simpson, K. L., *Phytochemistry* **6,** 1527 (1967); **9,** 1239 (1970).
(182) Recommended Dietary Allowances, NAS–NRC, Food and Nutrition, Board Publication 1964, 7th Ed. (1968).
(183) Roels, O. A., Symposium on Vitamin A, Ass. Vitamin Chem., Chicago, Feb 29, 1968.
(184) Roels, O. A., *Vitamins* **1,** 167 (1967).
(185) Rubin, S. H., De Ritter, E., *Vitam. Horm. (New York)* **12,** 101 (1954).
(186) Rüegg, R., Montavon, M., Ryser, G., Saucy, G., Schwieter, U., Isler, O., *Helv. Chim. Acta* **42,** 854 (1959).
(187) Salminen, K., Karinpää, A., Koivistoinën, P., Mukula, J., *Acta Agr. Scand.* **20,** 49 (1970).
(188) Saperstein, S., Starr, M. P., *Biochem. J.* **57,** 273 (1966).
(189) Savolainen, J. E. T., Gyllenberg, H. G., *Lebensm. Wiss. Technol.* **3,** 18 (1970).
(190) Schettino, O., Di Lieto, A., *Tec. Molitoria* **19,** 557 (1968).
(191) Schwieter, U., Isler, O., *Vitamins* **1,** 5 (1967).
(192) Scrimshaw, N. S., Symp., Prospects of World Food Supply, Nat. Acad. Sci., Washington, D. C. (1966).
(193) Scott, G. C., Belkengren, R. O., *J. Food Res.* **9,** 371 (1944).
(194) Scott, M. L., Ascarelli, I., Olson, G., *Poultry Sci.* **47,** 863 (1968).
(195) Searles, S. K., Armstrong, J. G., *J. Dairy Sci.* **53,** 150 (1970).
(196) Shrmizu, T., Oda, A., *Bull. Jap. Soc. Sci. Fish.* **34,** 627 (1968).
(197) Simonnet, H., *Wiss. Veroeff. Deut. Ges. Ernaehr.* **9,** 205 (1963).
(198) Simpson, K. L., Goodwin, T. W., *Phytochemistry* **4,** 193 (1965).
(199) Simpson, K. L., Nakayama, T. O. M., Chichester, C. O., *Biochem. J.* **92,** 508 (1964).
(200) Slanetz, C. A., Scharf, A., *J. Nutr.* **30,** 239 (1945).
(201) Steenbock, H., *Science* **50,** 352 (1919).
(202) Steinegger, P., Zanetti, G., *Arch. Gefluegelk.* **21,** 236 (1957); **23,** 166 (1959).
(203) Stephen, T. S., McLemore, T. A., *Food Technol.* **23,** 1600 (1969).
(204) Strachan, C. C., Moyls, A. W., Atkinson, F. E., Britton, J. E., *Can. Dep. Agr. Publ.* No. 862 (1951).
(205) Strain, H. H., Svec. W. A., *Advan. Chromatogr.* **8,** 119 (1969).
(206) Subbarayan, C., Lakshmanan, M. R., Cama, H. R., *Biochem. J.* **99,** 308 (1966).
(207) Surmatis, J. D., Maricq, J., Ofner, A., *J. Org. Chem.* **23,** 157 (1958).
(208) Surmatis, J. D., Walser, A., Gibas, J., Schwieter, U., Thommen, R., *Helv. Chim. Acta* **53,** 974 (1970).
(209) Suryanarayana, R. K., Rukmini, C., Mohan, V. S., *Ind. J. Agr. Sci.* **38,** 368 (1968).
(210) Sweeney, J. P., Marsh, D. E., *J.A.O.A.C.* **53,** 937 (1970).
(211) Tagwerker, F. J., Streiff, K., Brubacker, G., Proc. 12th World's Poultry Congress, Sydney, Australia (1966).
(212) Thommen, H., *Int. Z. Vitaminforsch.* **37,** 175 (1967).
(213) Thommen, H., *Naturwissenschaften* **51,** 87 (1964).
(214) Thommen, H., *Naturwissenschaften* **22,** 517 (1962).
(215) Thommen, H., Gloor, U., *Naturwissenschaften* **52,** 161 (1965).
(216) Thommen, H., Wiss, O., *Z. Ernaehr.* **3,** 18 (1963).
(217) Thompson, C. R., *Agron. J.* **41,** 294 (1949).
(218) Thompson, S. Y., *Proc. Nutr. Soc. Engl. Scot.* **24,** 136 (1965).
(219) Thompson, S. Y., *Exp. Eye Res.* **3,** 392 (1964).
(220) Thompson, S. Y., *Wiss. Veroeff. Deut. Ges. Ernaehr.* 9, 265 (1963).
(221) Tichenor, D. A., Martin, D. C., Wells, C. E., *Food Technol.* **19,** 106 (1965).
(222) Tiews, J., *Wiss. Veroeff. Deut. Ges. Ernaehr.* **2,** 235 (1963).
(223) Vander Cook, C. E., Yokoyama, H. J., *Food Sci.* **30,** 865 (1965).
(224) Viulleumier, J. P., *Poultry Sci.* **48,** 767 (1969).
(225) Washburn, R. G., Hibbs, J. W., Krause, W. E., *Ohio Agr. Exp. Sta. Res. Circ.* 8 (1960).
(226) Watt, B. K., Merrill, A. L., Composition of Foods, USDA–ARS Agr. Handbook No. 8 (1963).
(227) Weckel, K. G., Santos, B., Herman, E., Laferriere, L., Gabelman, W. H., *Food Technol.* **16,** 91 (1962).
(228) Weedon, B. C. L., *Fortsch. Chem. Org. Naturst.* **27,** 81 (1969).
(229) Weedon, B. C. L., Chemistry and Biochemistry of Plant Pigments, T. W. Goodwin, Academic Press, 1965, p 143.
(230) White, J. W., Jr., Zscheile, F. P., Brunson, A. M., *J. Amer. Chem. Soc.* **64,** 2603 (1942).
(231) Wildfeuer, I., *Z. Lebensm. Unters. Forsch.* **140,** 140 (1969).
(232) Williams, R. J. H., Britton, G., Goodwin, T. W., *Biochem. J.* **105,** 99 (1967).
(233) Winterstein, A., *Z. Physiol. Chem.* **215,** 51 (1933); **221,** 117, 129 (1933).
(234) Winterstein, A., *Angew. Chem.* **72,** 902 (1960).
(235) Winterstein, A., Studer, A., Rüegg, A., *Chem. Ber.* **93,** 2951 (1960).
(236) Witschi, J. C., Houser, H. B., Littell, A. S., *J. Amer. Diet. Ass.* **57,** 13 (1970).
(237) Yokoyama, H., White, M. J., *Phytochemistry* **7,** 1031 (1968); **9,** 231 (1970).
(238) Yokoyama, H., White, M. J., *J. Org. Chem.* **30,** 3994 (1965).
(239) Yokoyama, H., White, M. J., *J. Org. Chem.* **30,** 2481 (1965).
(240) Zechmeister, L., Cis–Trans Isomeric Carotenoids, Academic Press (1962).
(241) Zechmeister, L., Petracek, F. J., *Arch. Biochem. Biophys.* **61,** 243 (1956).
(242) Zechmeister, L., Tuzson, P., *Ber.* **67,** 824 (1934).
(243) Zubeckis, E., *Can. J. Publ. Health* **59,** 193 (1968).

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