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METABOLIC FATE OF FOOD COLORANTS

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INTRODUCTION

Common experience demonstrates that people desire and prefer foods that have appealing and characteristic colors. Color of foods may be due to natural pigments, e.g., carotenoids, chlorophylls, myoglobins, and anthocyanins; chemical modification during processing of natural constituents of foods, e.g. caramelization; and color additives. Processing conditions required for cooking and preserving foods in home or industry often result in color fade and require color additives to restore original appearance and appeal. In addition, foods and beverages may be prepared by combining

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individual chemical ingredients of both natural and synthetic origin, including color additives. Hence, exposure of the general population to color additives is ubiquitous.

In fiscal year 1979 the United States Food and Drug Administration (FDA) certified 5.87 million pounds of Food, Drug, and Cosmetic (FD&C)[1] primary color additives and 1.73 million pounds of FD&C lakes, water-insoluble salts of the primary colors. Certification of a batch of colorant indicates its conformance with chemical specifications issued by the FDA as part of the regulation permitting use of the colorant. Certification is generally applied to synthetic colorants. Natural colorants, whether derived from natural sources or synthesized de novo, e.g. carotenoids, are exempt from certification. The legislative and regulatory history of color additives has been reviewed recently by Noonan & Meggos (83). Food colorants are used typically in concentrations of 10–500 ppm in foods and beverages (25a, 48). One estimate of daily per capita consumption (disappearance) was about 54 mg (48).

In this review of recent studies of the metabolic fate of both natural and synthetic food colorants and their lakes, the primary emphasis is on colorants presently permitted in foods in the United States. Colorants previously used in the United States, those permitted in other countries, and new, experimental colorants are cited as examples that demonstrate unusual chemical properties, functionality, or particular public concern. The chemistry and stability of each class of colorant are described, since the compounds may be altered during food processing and storage prior to ingestion. Finally, toxicity and other biological effects are considered. The toxicology of food colors has been reviewed recently (30, 61, 89).

SYNTHETIC COLORANTS

Seven synthetic colorants are permitted by the FDA for general use in foods and in orally ingested drugs and cosmetics: FD&C Red No. 40, FD&C Red No. 3, FD&C Yellow No. 5, FD&C Yellow No. 6, FD&C Blue No. 1, FD&C Blue No. 2, and FD&C Green No. 3. Citrus Red No. 2 is permitted only for coloring skins of oranges and Orange B is permitted only for coloring the casings or surfaces of frankfurters and sausages. These compounds represent four distinct chemical classes (Table 1 and Figure 1), with each sharing the common property of water solubility conferred by one or more sulfonic or carboxylic acid groups. Sulfonation decreases fat solubility and also appears to decrease toxicity of related compounds within a class by enhancing urinary excretion of the dye and its metabolites.

[1]FD&C color additives are permitted by the FDA for use in foods, orally ingested drugs, and cosmetics applied to the lips or any body surface covered by mucous membrane.

Table 1 Identification of some important synthetic food colorants

Common name	Class	C.I. no. (EEC no.)	Structure[a]	Synonym(s)	Chemical Abstracts name (CAS reg. no.)
Amaranth	azo	16185 (E123)	I	FD&C Red No. 2	3-Hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-2,7-naphthalenedisulfonic acid, trisodium salt (915–67–3)
Allura red	azo	16035	II	FD&C Red No. 40, Food Red 17	6-Hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid, disodium salt (66813–73–8)
Carmoisine	azo	14720	III	Food Red 3, azorubine	4-Hydroxy-3-[(4-sulfo-1-naphthalenyl)azo]-1-naphthalenesulfonic acid, disodium salt (3567–69–9)
Citrus Red No. 2	azo	12156	IV	—	1-[(2,5-Dimethoxyphenyl)azo]-2-naphthol (6358–53–8)
Orange B	azo	19235	V	—	5-Hydroxy-4[(4-sulfo-1-naphthalenyl)azo]-1-(4-sulfophenyl)-(4-sulfophenyl)-pyrazole-3-carboxylic acid ethyl ester, disodium salt (53060–70–1)
Ponceau SX	azo	14700	VI	FD&C Red No. 4, Food Red 1	3-[(2,4-Dimethyl-5-sulfophenyl)azo]-4-hydroxy-1-naphthalenesulfonic acid, disodium salt (4548–53–2)
Ponceau 4R	azo	16255 (E124)	VII	Food Red 7, new coccine	7-Hydroxy-8-[(4-sulfo-1-naphthalenyl)azo]-1,3-naphthalenedisulfonic acid, trisodium salt (2611–82–7)

Sunset yellow	azo	15985 (E110)	VIII	FD&C Yellow No. 6	6-Hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid, disodium salt (2783–94–0)
Tartrazine	azo	19140 (E102)	IX	FD&C Yellow No. 5	4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)azo]-pyrazole-3-carboxylic acid, trisodium salt (1934–21–0)
Brilliant Blue FCF	triphenyl-methane	42090	X	FD&C Blue No. 1	*N*-Ethyl-*N*-(4-[(4-ethyl[(3-sulfophenyl)methyl] amino)phenyl] (2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene)-3-sulfobenzenemethaminiumhydroxide, inner salt, disodium salt (3844–45–9)
Fast Green FCF	triphenyl-methane	42053	XI	FD&C Green No. 3	*N*-(4-[(5-hydroxy-2,4-disulfophenyl) (4-{[(4-sulfophenyl) methyl]amino}phenyl) -methylene}-2,5-cyclohexadien-1-ylidene)-*N*-methylbenzenemethaminiumhydroxide, inner salt, disodium salt (12777–77–4)
Erythrosine	xanthene	45430 (E127)	XII	FD&C Red No. 3	2′,4′,5′,7′-Tetraiodofluoroscein, disodium salt (16423–68–0)
Indigotine	indigoid	73015 (E132)	XIII	FD&C Blue No. 2, indigo carmine	3,3′-Dioxo-(Δ2,2′-biindoline)-5,5′-disulfonic acid, disodium salt (860–22–0)
Quinoline yellow	quinoline	47005 (E104)	XIV	D&C Yellow No. 10, Food Yellow 13	2-(2-Quinolyl)-1,3-indandione, disodium salt (8004–92–0)

[a] See Figure 1 (see also 66).

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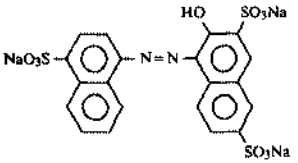

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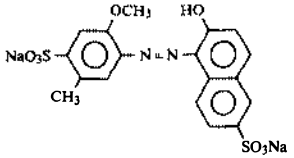

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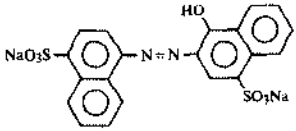

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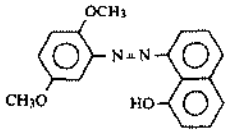

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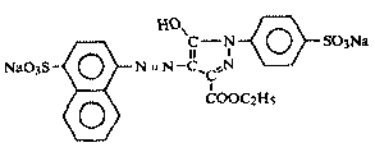

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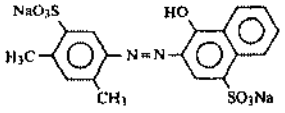

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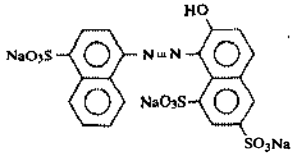

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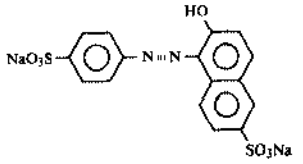

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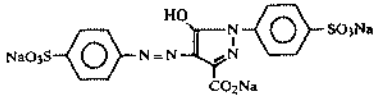

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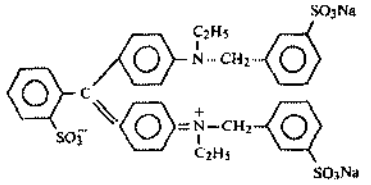

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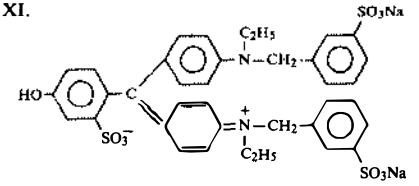

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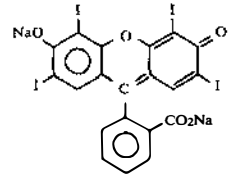

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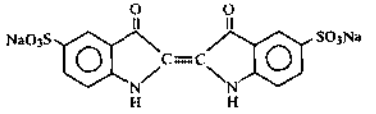

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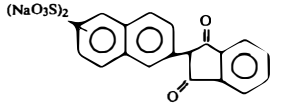

Figure 1 Structures of some important synthetic food colorants. Roman numerals refer to structures listed in Table 1.

Azo Dyes

CHEMISTRY AND STABILITY The azo dyes are mono-, di-, and trisulfonated compounds that contain a naphthalene or pyrazolone ring linked by an azo bond to a second naphthalene or a benzene ring. The azo bond can be reduced in foods and beverages, forming amine cleavage products. Reduction, resulting in color fade, can be pronounced in carbonated and still beverages, especially in the presence of ascorbic acid. The reductive action of ascorbic acid is enhanced by sunlight; significant protection can be obtained by packaging such beverages in cans rather than in colorless bottles. The azo bond also is reduced by anaerobic intestinal microflora (13). Cleavage occurs largely extracellularly and appears to be mediated by a redox shuttle that requires bacterial intracellular enzymes and extracellular mediators such as free flavins (13, 21, 24). Reductive cleavage of sunset yellow and tartrazine was not diminished by attaching the dyes to a high-molecular-weight polyaminoethylene backbone (12, 21, 51). Rates of reduction of amaranth, sunset yellow, new coccine, and tartrazine by suspensions of human fecal microorganisms did not differ markedly among fecal samples from individual subjects in spite of considerable variations in age, daily diet, and living circumstances (114).

METABOLISM AND TOXICITY The highly sulfonated, intact azo dyes are poorly absorbed from the intestine after oral administration (112). Reductive cleavage products formed in situ by intestinal bacteria are rapidly and extensively absorbed, further modified by the liver, and excreted in the bile and urine (112). Hence, study of the biological effects of orally administered azo dyes is predominantly the study of the toxicity of their metabolites. Toxicity associated with intravenous or intraperitoneal administration of azo dyes may be irrelevant to their use as food colorants.

Classic studies made in the 1960s of absorption, metabolism, and excretion of azo food dyes have been reviewed (112). These studies identified the cleavage products of the major dyes then in use and described their metabolic fate in various species (Table 2). Recent metabolic studies that used more sensitive analytical chemical methods or radiolabeled compounds have largely confirmed the earlier findings, provided more accurate pharmacokinetic data under various experimental conditions, and identified additional minor metabolites. Thus, Honohan et al (51) found when ^{14}C-labeled sunset yellow was administered orally to rats, urinary excretion of the two cleavage products, sulfanilic acid and 1-amino-2-naphthol-6-sulfonic acid, was 40 and 8.5% of the theoretical molar equivalents of each, respectively. They found only traces of intact dye in the feces of rats after oral administration of ^{14}C-labeled tartrazine, indicating apparently complete cleavage in the gastrointestinal tract.

Amaranth (FD&C Red No. 2) is cleaved by intestinal microflora to 1-amino-4-naphthalene sulfonic acid (naphthionic acid) and 1-amino-2-hydroxy-3,6-naphthalenedisulfonic acid, both of which are absorbed from the intestine. Following a single oral dose of amaranth to fasted rats, serum naphthionic acid concentration reached a maximum 2 h after dosing and remained constant for another 6 h (88). In fed animals, peak serum levels were approximately one half those in fasted rats. Multiple dosing by gavage at 24-h intervals or administration in the drinking water resulted in a cyclic pattern of serum napthionic acid levels. Consumption of amaranth in the diet resulted in constant serum naphtionic acid levels at a maximum level equivalent to that seen with the other routes of administration. This difference in exposure to a major amaranth metabolite is important in interpreting toxicity data and their relevance to human exposure to the colorant in foods and beverages.

Ruddick et al (90) found radioactive amaranth, naphthionic acid, and up to five unidentified metabolites in the gastrointestinal tract contents, urine, and feces after administering an oral dose of ^{14}C-labeled amaranth to fasted rats. Consumption of food immediately after dosing resulted in generally lower tissue:blood radioactivity concentration ratios, lower blood radioactivity levels, and a lower percentage of retention of radioactivity in tissues. Decrease in formation of amaranth metabolites or absorption of metabolites and intact dye may explain the observed protective effects in rats of various dietary fibers against amaranth toxicity (35, 102).

When amaranth was fed at 200 and 2000 mg/kg [body weight (bw)] to female rats throughout mating and gestation, maternal plasma naphthionic acid levels were about 5-fold higher than were fetal plasma levels (117). Maternal plasma naphthionic acid levels increased 7.7-fold with a 10-fold increase in dye intake, whereas fetal plasma levels increased 5.5-fold. These results showed napthionic acid does not freely cross the placenta and that maternal placental transfer processes are at least partially saturated at a feeding level of 2000 mg/kg.

Amaranth was one of the first seven color additives approved for use in foods and drugs in the U.S. by the Food and Drug Act of 1906, and it enjoyed a long history of apparent safe use. However, in 1970, questions were raised about its possible oncogenic and reproductive effects as a result of studies conducted in the Soviet Union. A subsequent study by the FDA was inconclusive because of faulty execution (8). In January 1976, the provisional listing of FD&C Red No. 2 (amaranth) was terminated and several weeks later the petition for permanent listing was denied on the basis that its safety had not been established (7, 36). This conclusion was not accepted by regulatory agencies elsewhere and amaranth continues to be one of the most widely used synthetic red colorants throughout the world. The toxicology of amaranth has been reviewed thoroughly (30, 56, 61).

Ironically, most foreign countries have not approved the use of allura red (FD&C Red No. 40), which replaced amaranth as the dominant red food colorant in the U.S. (25). Unpublished reports of the metabolism and toxicology of allura red were reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974 (55). A 92-week study in rats at a maximum dietary level of 5.2% showed no effect other than moderate growth depression. However, the number of animals per group (30 males and 30 females) and their survival rates were considered inadequate and JECFA concluded that it was not possible to allocate an acceptable daily intake (ADI) from the available limited information. In 1979, JECFA reviewed additional studies, including two lifetime mouse studies, and concluded allura red did not have carcinogenic potential (59). An ADI of 0–7 mg/kg (bw) was established. In 1980, JECFA said this should be considered temporary pending statistical reanalysis of two chronic mouse studies by the FDA. A final decision on FD&C Red No. 40's regulatory status is expected in 1981.

None of the currently permitted FD&C dyes are mutagenic in the Ames *Salmonella*/microsome test (3, 20, 40). However, other azo dyes that are nonmutagenic per se are active after chemical or bacterial reduction and metabolic activation (20, 23, 47, 101). The variety of these treatment conditions required to yield specific mutagenic responses suggests that other more suitable test modifications might be developed for evaluating the potential genotoxicity of azo food dyes and their relatively unstable primary cleavage products.

Triphenylmethane Dyes

Triphenylmethane dyes (FD&C Blue No. 1, FD&C Green No. 3) are not widely used by themselves in foods, but are important in blends with other dyes to achieve purple and green hues (48).

CHEMISTRY AND STABILITY Like azo dyes, triphenylmethane dyes are easily reduced to colorless forms in food, but do not undergo reductive cleavage. Contact with metal surfaces (zinc, tin, aluminum, and copper) previously was important in color fading, but advances in food processing have decreased contact with these metals to a minimum. Reducing agents such as ascorbic acid enhance fading.

METABOLISM AND TOXICITY Studies with spectrophotometric analyses have demonstrated intestinal absorption of less than 10% and rapid biliary excretion of oral doses of triphenylmethane dyes, including FD&C Blue No. 1 and FD&C Green No. 3 (47, 79, 80). Two recent studies with

Table 2 Azo bond cleavage products of some azo food colorants

Common name	Cleavage products
Amaranth	1-amino-4-naphthalenesulfonic acid (naphthionic acid), 1-amino-2-hydroxy-3,6-naphthalenedisulfonic acid
Allura red	1-amino-2-hydroxy-7-naphthalenesulfonic acid, 1-amino-2-methoxy-5-methyl-4-benzenesulfonic acid
Carmoisine	naphthionic acid, 1-amino-2-hydroxy-7-naphthalenesulfonic acid
Citrus Red No. 2	1-amino-2-hydroxynaphthalene, 1-amino-3,6-dimethoxybenzene
Orange B	naphthionic acid, 1-(4-sulfophenyl)-3-carboxy-4-amino-5-pyrazolone, ethyl ester
Ponceau SX	1-amino-2-hydroxy-7-naphthalenesulfonic acid, 1-amino-2,4-dimethyl-5-benzenesulfonic acid
Ponceau 4R	naphthionic acid, 1-amino-2-hydroxy-6,8-naphthalenedisulfonic acid
Sunset Yellow	1-amino-4-benzenesulfonic acid (sulfanilic acid), 1-amino-2-hydroxy-6-naphthalenesulfonic acid
Tartrazine	sulfanilic acid, 1-(4-sulfophenyl)-3-carboxy-4-amino-5-pyrazolone

^{14}C-labeled FD&C Blue No. 1 (Brilliant Blue FCF) and ^{14}C-labeled Green S (C. I. 44090) have confirmed their low level of absorption and provide additional details of metabolism. Brown et al (18) found intestinal absorption of ^{14}C-labeled FD&C Blue No. 1 in bile duct-ligated rats (estimated from 96-h urinary ^{14}C excretion, expired $^{14}CO_2$, and residual tissue radioactivity) was 2.05% of a 1.5 mg/kg oral dose. Fecal excretion was 97.3%. In intact rats, absorption was estimated to be 0.3% and fecal excretion was 91.1%, whereas biliary excretion in bile duct-cannulated rats was 1.3%. Thin-layer chromatography of bile and urine showed 95% of excreted radioactivity was unchanged dye and 5% was an unidentified desulfonated metabolite or degradation product. Phillips et al (87) found less than 2% of 0.1 and 10 mg/kg oral doses of ^{14}C-labeled Green S and less than 1% of 0.03 and 3 mg/kg doses of ^{14}C-labeled FD&C Blue No. 1 in 72-h urines of rats, mice, and guinea pigs. Absorption in rats was not enhanced by prefeeding with nonlabeled dye for 21 days. Low levels of absorption were confirmed in isolated intestinal loops in situ. Thin-layer chromatography of fecal extracts showed a distribution of label similar to that of the original dye.

The low degree of toxicity of triphenylmethane dyes seen in chronic feeding studies is ascribed to their low level of intestinal absorption (30, 55, 61). FD&C Blue No. 1 and FD&C Green No. 3 were not mutagenic in the Ames *Salmonella*/microsome test (3, 20) or in *Bacillus subtilis* (31).

Xanthene Dyes

CEMISTRY AND STABILITY Erythrosine is the only xanthene derivative currently permitted in foods in the U.S. and European Economic Community (Common Market) (EEC) countries. The dye has poor light stability, which limits its application in coatings and beverages; it is used principally in confections, dessert powders, and baked goods (48). Erythrosine is 58% iodine by weight; fluorescein and free iodine may be produced under some processing and storage conditions (29).

METABOLISM AND TOXICITY Erythrosine is poorly absorbed from the gastrointestinal tract in rats (26, 116). Absorbed dye is excreted in bile intact and in deiodinated forms, but no degradation of the fluorescein nucleus occurs (53, 116). In rats fed erythrosine-colored cereal diets containing 5.2–13.9 μg of iodine/g of diet for 3–5 weeks, significant deiodination of the dye was demonstrated by decrease in thyroid ^{131}I uptake and serum PB ^{131}I following intraperitoneal injections of Na ^{131}I (109). However, in an acute study in rats, deiodination was not seen (99). Absorption of erythrosine from colored pharmaceutical preparations had led to false elevations of serum protein-bound iodine values in man, which were found to be due to interference by the circulating dye with the protein-bound iodine measurement (2, 10, 45). Although deiodination appears to be an important catabolic pathway of erythrosine, its exact metabolic fate in man has not been determined.

Erythrosine was one of the seven original colors permitted by the U.S. Food and Drug Act of 1906. Results of toxicological studies with erythrosine in mice, rats, gerbils, guinea pigs, rabbits, dogs, and pigs have been evaluated by JECFA (55). A no-effect level of 250 mg/kg (bw) was determined in rats and the ADI in man was estimated to be 2.5 mg/kg (bw).

Erythrosine increased the mutation rate in streptomycin-dependent *Escherichia coli* (74). However, the dye was not mutagenic in the Ames *Salmonella*/microsome test (3, 20). Four xanthene dyes (erythrosine, phloxin, rose bengal, and acid red) were most toxic among 11 food dyes permitted in Japan that were tested on cultured fetal rat hepatocytes (91).

Erythrosine has been reported to inhibit neurotransmitter uptake in crude rat synaptosomes (65) and brain homogenates (72). However, Mailman et al (75) have hypothesized that these effects in vitro might result from nonspecific interactions with neural membranes (70).

Indigoid Dyes

CHEMISTRY AND STABILITY FD&C Blue No. 2 is unstable in aqueous solution and is readily oxidized to isatin-5-sulfonic acid and 5-sulfoan-

thranilic acid (60). Nonionic surfactants and sugars increase rate of fading (64, 95).

METABOLISM AND TOXICITY Three days after oral administration of ^{35}S-labeled FD&C Blue No. 2 to rats, less than 3% of the radioactivity appeared in urine as the unchanged dye and its two oxidative products (69). Amounts in feces were due to unabsorbed dye rather than to biliary excretion. After oral administration of 5-sulfoanthranilic acid, 24% appeared in urine in 2 days. Indigotine was one of the seven original colors permitted by the U.S. Food and Drug Act of 1906. JECFA (55) reviewed the extensive toxicity testing of indigotine and estimated a no-effect level of 500 mg/kg (bw) in the rat and the ADI in man as 0–5 mg/kg. Indigotine was not mutagenic in cultures of *E. coli* (73) or in the Ames *Salmonella*/microsome test (3, 20).

Quinoline Dyes

Quinoline yellow (D&C Yellow No. 10) is provisionally listed in the U.S. for use in orally ingested drugs and cosmetics applied to the lips and mucous membrane surfaces. It is approved for use in foods and beverages in EEC countries. Its future use in foods and beverages in the U.S. as a possible substitute for tartrazine depends on the outcome of chronic rodent toxicity studies now in progress.

METABOLISM AND TOXICITY Relatively little is known about the metabolic fate of quinoline yellow. After oral administration to rats, 2% of the dose appeared in the urine and 97% appeared in the feces in 48 h (111). Excretion was essentially complete in 24 h. The low level of intestinal absorption was confirmed in anesthetized bile duct-cannulated animals; about 1% of the dose was recovered in bile and urine after 4-5 h. Quinoline yellow was not metabolized by rat cecal contents in vitro and did not affect hepatic drug metabolizing enzymes (111).

JECFA reviewed the toxicology of quinoline yellow (57) and determined a no-effect level in the rat of 50 mg/kg (bw). A temporary ADI of 0–0.5 mg/kg (bw) was estimated from the data, pending result of metabolic fate studies in several species, including man; adequate chronic testing in an additional species; and a multi-generation reproduction study. Quinoline yellow was not mutagenic in *E. coli* (73) or in the Ames *Salmonella*/microsome test (J. P. Brown & P. S. Dietrich, unpublished results).

Polymeric Dyes

In an attempt to minimize intestinal absorption and metabolism of food colorants, and thereby decrease their potential toxicity, polymeric dyes have

been synthesized that retain the essential properties of their monomeric equivalents but are chemically and biologically more stable (39, 111a).

CHEMISTRY AND STABILITY Early prototypes were polymeric azo dyes prepared from poly (vinylamine hydrochloride) (27, 51). Polymeric derivatives of sunsent yellow and tartrazine with molecular weights of 125,000 and 59,000, respectively, had desirable spectral properties and chemical stability, but were metabolically unstable (51, 83). These were replaced in development by dyes utilizing stable, water-insoluble anthraquinone and anthrapyridone chromophores (red) on soluble vinylamine-vinylsulfonate copolymers (28) or water-soluble nitrobenzenesulfonic acid chromophores (yellow) on poly(vinylamine). These compounds were selected on the basis of the physical and chemical properties of color, solubility, thermal stability, light stability, and compatibility with other food ingredients (6, 111a). Polymeric dyes, singly or in blends, give metameric color matches relative to target shades containing FD&C dyes and exhibit color stability throughout the pH 2.4–8.0 range typical of most food products. Their solubility in water is limited by the viscosity of the solution to about 20% by weight. Thermal stress under a wide range of conditions showed less than 0.5% chromophore cleavage and the absence of polymer chain scission. Comparative light stability studies showed polymeric dyes to be more photochemically stable than amaranth, allura red, and tartrazine in a commercial carbonated beverage and in a model still beverage (6).

METABOLISM AND TOXICITY Polymeric derivatives of sunset yellow and tartrazine were cleaved at the azo bond by intestinal microflora to the same extent as their monomeric equivalents (12, 21, 51). In rats dosed by gavage with sunset yellow and its polymer derivative, absorption of the cleavage product 1-amino-2-naphthol-6-sulfonic acid was 8.5 and 6.9%, respectively (51). In rats dosed with tartrazine and its polymeric derivative, absorption of the cleavage product aminopyrazolone and its metabolites was 4.0 and 4.6%, respectively. The sulfanilic acid moiety of both dyes remained attached to the polymer backbone and was not absorbed.

In contrast to the polymeric azo dyes, a red polymeric colorant utilizing an anthrapyridone chromophore (Poly R-478) was stable in the gastrointestinal tract (15). Intestinal absorption following oral administration of ^{14}C-labeled Poly R-478 was less than 0.5% in mice and rats (39), guinea pigs and rabbits (T. M. Parkinson, unpublished results), and man (113). Similar results have been found in absorption studies with other non-azo polymeric dyes (Poly R-481, a red; and Poly Y-607, a yellow) in mice, rats, guinea pigs, and rabbits (83), and in man (P. D. Walson, D. E. Carter, B. A. Ryerson, D. Clark, & T. M. Parkinson, unpublished results). Urinary

excretion of radioactivity could be accounted for by small amounts of unattached ^{14}C-labeled chromophore present in the administered dyes.

Effects of Poly R-478 on lower bowel flora were evaluated in rats fed the dye at a dietary level of 5% by weight for 90 days (15). A battery of 15 selective and nonselective media used to measure anaerobic and facultatively anaerobic flora showed no biologically significant changes in their concentrations. Poly R-478 is considered inert with respect to gut flora in the rat.

Poly R-481 and Poly Y-607 are nontoxic at single oral doses of 10 g/kg (bw) in mice, rats, and dogs and at 5% by weight in the diet in 13-week feeding studies in rats (83). Chronic feeding studies are in progress. The polymeric dyes have been evaluated extensively in bacterial, yeast, and mammalian genotoxicity tests (19) and have been found to be nonmutagenic (J. P. Brown, unpublished results).

Lakes

FD&C aluminum lakes are insoluble pigments made from certified FD&C dyes by adsorbing them on an aluminum hydroxide (alumina hydrate) substrate with a basic aluminum or calcium radical (32). The certified FD&C lakes may be used in foods or any materials that might come in contact with food and be ingested, e.g. printing inks, paper, or plastics. Pure dye content of standard lakes is approximately 10–40%. Physical and chemical properties of FD&C lakes are summarized by Dunn & Steinbach (32). Polymeric dyes form lakes with unusually high dye content with greater bleed resistance in aqueous systems (83).

It is believed that the alumina substrate dissolves in the stomach, liberating water-soluble dye. Consequently, no additional metabolism or toxicity studies are required. The adsorbed dyes would not be expected to be extensively metabolized, but small amounts could be released during food processing or storage, or in the gastrointestinal tract after ingestion. Since the particle sizes of lakes are smaller than 1 μm, absorption of the intact pigment is possible (68).

NATURAL COLORANTS

Anthraquinones

Carminic acid (Figure 2) and its aglycone kermesic acid are anthraquinones from the cochineal insect (*Dactylopius coccus*) and the kermes insect (*Kermococcus vermilius*), respectively. The biology and historical significance of these dyes have been discussed recently by Eisner et al (32a) and Baranyovits (5a). The term cochineal refers to red coloring material consisting of dried crushed bodies of female *D. coccus,* whereas carmine refers to the aluminum lake of the active principle, carminic acid, that has been

extracted from the insect. The pigment carmine can be solubilized by alkali for various applications (82).

The anthraquinone colors are characterized by relatively high chemical and biological stability. Although the structure of kermesic acid is similar to a number of phenolic anthraquinones and related mycotoxins that possess mutagenic, carcinogenic, or other toxic potential, no genotoxic effects have been noted to date in short-term studies with carminic acid (5, 14). Studies with a variety of anthraquinone compounds have shown that anthraquinone glyclosides in general are far less toxic than are their aglycones and that *C*-glycosides are more resistant to chemical or biological hydrolysis, e.g. by intestinal bacteria, than are the corresponding *O*-glycosides (16). Although no tests have been performed on kermesic acid per se, it seems possible that very little of this aglycone is generated following ingestion by man. Nevertheless, currently no data are available on the metabolic fate of carminic acid or on the effects of long-term feeding. The results of a recent three-generation study in rats, at daily dietary intakes of up to 500 mg/kg (bw), demonstrated that carmine had no untoward effects on the growth and fertility of adult rats, or in the pre- and post-natal development of their offspring (11). Two 90-day feeding studies were carried out in rats at doses up to 500 mg/kg (bw)/day. No obvious toxicity was noted in either study (55). A final chronic toxicity (carcinogenicity) study is in progress (11).

Anthocyanins

CHEMISTRY AND STABILITY The anthocyanins comprise a diverse group of glycosidic derivatives of the 2-phenylbenzophyrylium (flavylium) structure. They are intensely colored ampholytes and impart the blue, violet, and certain red colors to many edible fruits and berries. At pH 1.0 and below, anthocyanins exist as red flavylium salts; color gradually fades as the pH rises. At pH 4–5, the anthocyanins exist as colorless pseudobases. As the pH is raised above 5.0, they exist successively as purple quinoidal anhydro bases (pH <7), deep blue ionized anhydro bases (pH <8), and finally as yellow/brown chalcones (pH >12). Both high temperature and molecular oxygen exert deleterious effects on anthocyanins, leading to browning, precipitation, and color loss. Similarly, the presence of certain sugars, particularly fructose, or sulfur dioxide, can lead to loss of anthocyanin (34, 98). This chemical instability, coupled with high cost and low tinctorial strength, has limited the commercial application of anthocyanin pigments.

Most naturally occurring anthocyanins are 3-*O*-glycosides or 3,5-di-*O*-glycosides, the corresponding aglycones being termed anthocyanidins [Figure 2 (II)]. Six common anthocyanidins occur in edible plants (Table 3). A

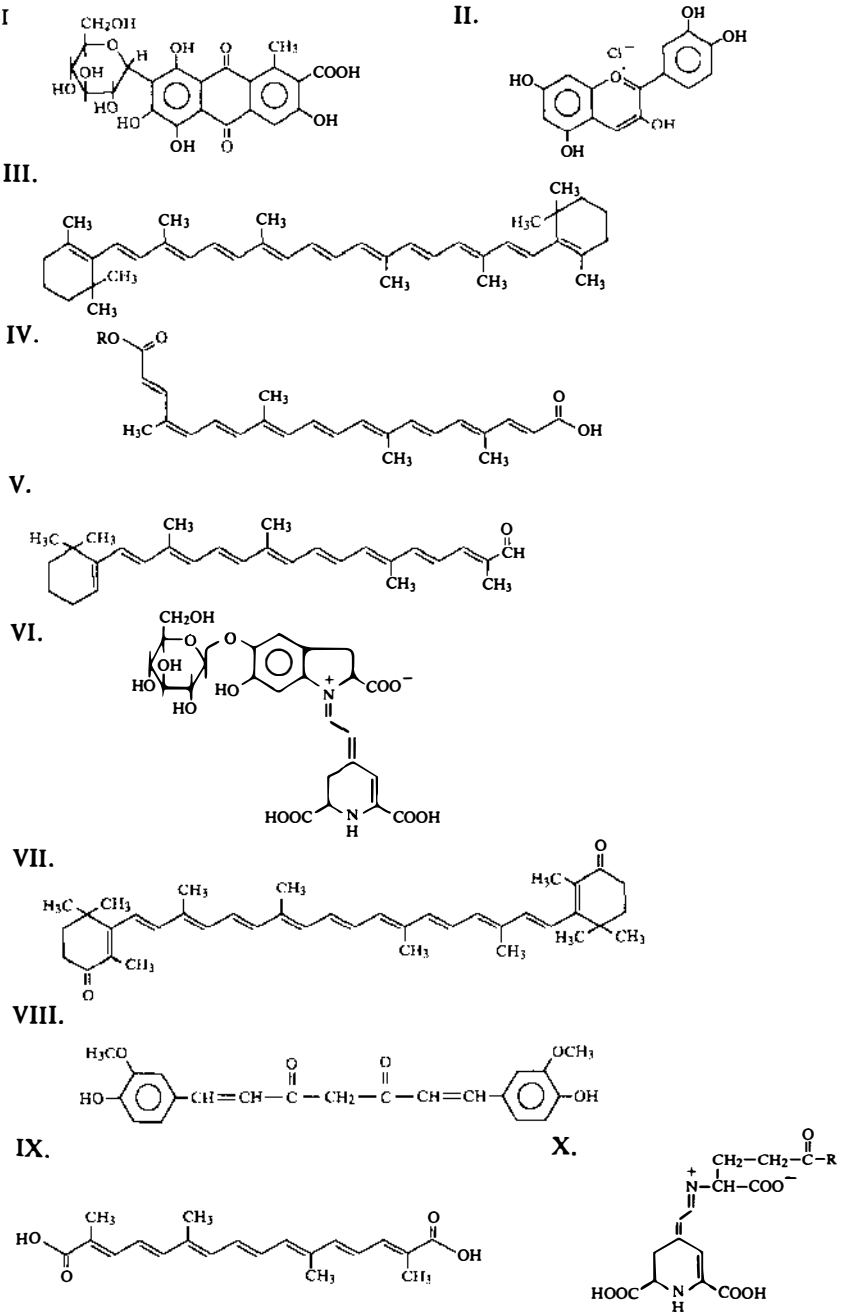

Figure 2 Structures of some important natural colorants ingested by man. Roman numerals refer to structures listed in Table 3.

Table 3 Identification of some important natural colorants ingested by man

Common name	Class	C.I. no. (EEC no.)	Structure	Synonyms	Systematic name(s)
Carminic acid	Anthraquinone	75470 E120	I $C_{22}H_{20}O_{13}$	Carmine, cochineal, C.I. Natural Red 4	7-α-D-Glucophranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2-anthacene carboxylic acid
Cyanidin	Anthocyanidins = aglycones; anthocyanin = various *O*-glycosides of 2-phenyl benzopyrylium salts, e.g. 3-monosides, 3-biosides, 3-triosides, 3,5- and 3,7-diglycosides	E163	II $C_{15}H_{11}ClO_6$	Grape skin extract	3,3′,4′,5,7-Pentahydroxy-2-phenyl benzopyrylium chloride
Pelargonidin					3,4′,5,7-Tetrahydroxy-2-phenyl benzo pyrylium chloride
Peonidin					3,4′,5,7-Tetrahydroxy-3′-methoxy-2-phenyl benzopyrylium chloride
Delphinidin					3,3′,4′,5,5′,7-Hexahydroxy-2-phenyl benzopyrylium chloride
Petunidin					3,4′,5,5′,7-Pentahydroxy-3′-methoxy-2-phenyl benzopyrylium chloride
Malvidin					3,4′,5,7-Tetrahydroxy-3′,5′-dimethoxy-2-phenyl benzopyrylium chloride
β-Carotene	Carotenoid	75130 E160a	III $C_{40}H_{56}$	DFGL Orange 3, Food Orange, C.I. Natural Yellow 26	
Bixin	Carotenoid	75120 E160b	IV $C_{25}H_{30}O_4$	Annatto extract, C.I. Natural Orange 4	6,6′-Diapo-4,4′-carotene dioic acid mono methyl ester (R = CH_3)
Norbixin			$C_{24}H_{28}O_4$	L-Orange No. 3, Butter color	6,6′-Diapo-4,4′-carotene dioic acid (R = H)

β-Apo-8-carotenal	Carotenoid	40820 E160e	V $C_{30}H_{40}O$	DFG Orange 8, Food Orange 6	β-Apo-2′-4-carotenal, L-β-apo-8′-carotenal, 8′-apo-β-4-caroten-8′-al
Capsanthin	Carotenoid	E160c	$C_{40}H_{56}O_3$	Paprika extract, paprika oleoresin	(3R,3′,5,5′ R)-3,3′-Dihydroxy-β,κ-carotene-6′-one
Crocetin	Carotenoid aglycone;	75100	IX $C_{20}H_{24}O_4$	Saffron, C.I. Natural Yellow 6	8,8′-Diapocarotene-8,8′-dioic acid, Digentiobiosyl-8,8′-diapocarotene-8,8′-dioate
Crocin	Carotenoid glycoside		$C_{44}H_{64}O_{26}$		
Canthaxanthin	Xanthophyll	40850 E161g	VII $C_{40}H_{52}O_2$	DFG L-Orange 7, Food Orange 8	4,4′-Diketo-β-carotene
Betanine	Betalaines	E162	VI $C_{24}H_{26}O_{13}N_2$	Beet red	
Vulgaxanthins	Betaxanthin		X[a]	Beet red	
Curcumin	—	75300 E100	VIII $C_{21}H_{20}O_6$	Turmeric, curcuma, Indian saffron, C.I. Natural Yellow 3	1,7 Bis(4-hydroxy-3-methoxy phenyl) hepta-1,6-diene-3,5-dione
Chlorophyllin copper complex KNa	Porphyrin	E141	$C_{34}H_{31}N_4$ $NaKCuO_5$	L-Grün No. 2	Copper complex of 1,3,5,8-tetramethyl-4-ethyl-2-vinyl-9-oxo-10 carboxy phorbin-7 propionic acid, Na,K salt
Riboflavin	Isoalloxazine	E101	$C_{17}H_{20}N_4O_6$	Vitamin B_2, lactoflavin, vitamin G	7,8-Dimethyl-10-(D-*ribo*-2,3,4,5-tetrahydroxypentyl) isoalloxazine

[a] Vulgaxanthin I, R = NH_2; vulgaxanthin II, R = OH. See Figure 2 (see also 67).

recent review by Kühnau (63) lists some 60 glycosidic combinations for anthocyanins in food plants and Timberlake & Bridle (104) list 16 naturally occurring anthocyanidins and 3-deoxyanthocyanidins; thus, the actual number of naturally occurring anthocyanins is probably on the order of 200.

METABOLISM AND TOXICITY Relatively little is known about the metabolic fate of anthocyanins despite their widespread occurrence and sizeable daily intake, estimated at 180–215 mg/day (63). The metabolism of anthocyanins and anthocyanidins has been studied in vivo in rats and in vitro with intestinal microorganisms. Scheline (93) incubated cyanidin chloride anaerobically with rat cecal bacteria for 22 h and found no detectable metabolites. Griffiths & Smith (43, 44) and Scheline (94) observed that pelargonidin was degraded by similar in vitro incubation to *p*-hydroxyphenyl lactic acid. The other ring fission product was presumably phloroglucinol (1,3,5-trihydroxy benzene). Delphinidin was administered to a rat intragastrically and an unidentified metabolite was found in the urine. Two metabolites were found in vitro by incubation with cecal microflora. Malvin, the 3,5-diglucoside of malvidin, underwent similar in vivo and in vitro tests. In the former, a number of metabolites, including syringic acid, were found in the urine. In the latter, none of the above metabolites were found with the exception of a trace of syringic acid.

It is unknown to what extent anthocyanins or their aglycones are absorbed following ingestion, although the studies mentioned above indicate that ring fission and glycoside hydrolysis occur to a limited extent with certain anthocyanins. Early studies with experimental animals indicated urinary excretion of less than 2% of oral anthocyanins from grapes (52, 94). Cyanidin, the most widespread anthocyanidin, is resistant to catabolism by intestinal bacteria, an unusual finding in view of its hydroxylation pattern, which, when present in other flavonoid compounds, e.g. flavonols, predisposes those compounds to bacterial ring fission.

Cyanidin and delphinidin have been tested for mutagenicity in the *Salmonella*/mammalian microsome assay and were found to be nonmutagenic (17). These findings are in sharp distinction to certain other flavonoids, namely, the flavonols (14).

Carotenoids and Xanthophylls

OCCURRENCE The carotenoids, aliphatic or alicyclic unsaturated terpenes composed of eight isoprene units, are the most widespread of natural colors in both the plant and animal kingdoms (30). At least 300 naturally occurring carotenoids are related to the parent compound, lycopene, the red pigment of tomatoes (25b, 34, 54), only a dozen of which are approved for

food use. With the exception of bixin, astaxanthin, and crocin, the carotenoids are oil-soluble colors. The xanthophylls comprise a group of yellow carotenoid pigments closely related to the carotenes but having keto and/or hydroxyl substituents. The most important commercial carotenoids are β-carotene, β-apo-8'-carotenal, and canthaxanthin. Colors from yellow to cherry can be obtained by blending two or more of these.

Carotenoid food colorants may be natural or synthetic. Natural extracts containing carotenoids have been used as food colors for centuries. Such natural extracts include annatto from the seeds of the tropical plant *Bixa orellana,* which contains bixin as the main component; saffron, which contains crocetin, β-carotene, and zeaxanthin; paprika extract (*Capsicum annum*), which contains capsanthin and capsorubin; xanthophyll extracts from leaves; carrot extracts with β- and α-carotenes; canthaxanthin from the pink edible mushroom *Cantharellus cinnabarina*; and red palm oil with lycopene and lutein. Details of the various applications of several natural carotenoids and their sources can be found in the articles of Emodi (33) and Engel (34). The synthetic carotenoids are chemically identical to the natural compounds, are of well-defined syntheses, and are prepared from essentially pure chemicals. Although many carotenoids have been synthesized, only β-carotene, apo-carotenal, and canthaxanthin are available in commercial quantities. Synthetic carotenoids have the advantage of higher purity and more uniform color than natural carotenoids. However, foods containing them must be labeled as containing artificial color. Also, the synthetic carotenoids have the same sensitivity to air, light, and temperature as the natural colors.

STABILITY Thermal isomerization, e.g. *trans*-β-carotene to 15,15'-mono-*cis*-β-carotene, is one of the changes that occurs during food processing that causes a color loss. A second type of alteration caused by heat, light, or radiation is 5,6-epoxide formation, which also results in color loss. On exposure to air, carotenoids slowly decompose as their conjugated double bonds oxidize. A variety of hydroperoxides and carbonyl compounds contribute to browning and off odors. Indirect oxidation can result from co-oxidation with unsaturated lipids such as linoleic acid. The presence of metal ions, particularly copper, catalyzes the degradation of carotenoids. Exposure to light promotes *cis*-isomerization in a manner similar to heat. Carotenoids are degraded by lipid-oxidizing enzymes such as peroxidases, lipoxidase, and lipoperoxidase, which may be present in certain plant extracts and tissues (86). Despite limitations imposed by oxidative chemical instability and high cost, the carotenoids enjoy many advantages, including relatively low toxicity, synthetic availability, highly desirable colors and high tinctorial strength, vitamin A activity, stability in the presence

of reducing agents (ascorbic acid), non-corrosivity, and antioxidant activity.

METABOLISM AND TOXICITY The toxicity of canthaxanthin, β-apo-8'-carotenal, β-carotene, and the ethyl and methyl esters of β-apo-8'-carotenoic acid has been reviewed by JECFA (55).

Canthaxanthin Canthaxanthin does not exhibit provitamin A activity. Acute oral toxicity in the mouse is low [$LD_{50} = 10$ g/kg (bw)]. Long-term feeding studies in the rat at up to 5% by weight in the diet for 98 weeks indicated no toxic effects.

Apo-carotenal Apo-carotenal, when administered to rats as the sole dietary carotenoid, is partially absorbed and converted to β-apo-8'-carotenoic acid and vitamin A (retinol). All three compounds accumulate in the liver. Unabsorbed apo-carotenal is excreted in the feces. In dogs, apo-carotenal is poorly absorbed from the gastrointestinal tract and is excreted together with apo-carotenoic acid in the urine. Only 4% of dietary carotenal is converted to vitamin A in the gut of vitamin A-deficient rats, compared with 10% of β-carotene. The acute toxicity of apo-carotenal in the mouse is very low, $LD_{50} > 10$ g/kg (bw). Subchronic administration to male rats of up to 500 mg of apo-carotenal/kg (bw)/day for 34 weeks resulted in no adverse effects. Testicular weights in the high-dose group were significantly lower than were the control, but this had no effect on fertility, as indicated by monthly mating of females. Groups of male and female dogs were administered up to 1.0 g of apo-carotenal/day for 14 weeks with no significant toxic effects or pathological lesions observed. A three-generation study in the rat at up to 5000 ppm in the diet for 2 years showed no adverse effects attributable to apo-carotenal (55).

Ethyl and methyl esters of β-apo-8'-carotenoic acid The ethyl and methyl esters of β-apo-8'-carotenoic acid have been less extensively studied, although these, along with the apocarotenoic acid, are normal metabolites of apo-carotenal. Only a small fraction of methyl apocarotenoate was absorbed if given in large doses to rats; the remainder was excreted in the feces. Acute toxicity in mice was very low, $LD_{50} > 10$ g/kg (bw). A subchronic toxicity study in rats fed up to 500 mg of methyl ester/kg (bw)/day for 34 weeks revealed no effects except reduced testicular weights in the high-dose group. Spermatogenesis was not affected. In a four-generation study, methyl ester was fed to male and female groups at up to 1.0% of the diet for 52 to 104 weeks. No toxic effects or pathology was observed. In a second study involving male rats, the ethyl ester was fed at 1% in the diet for 2 years.

Mortality and other test parameters were identical in test and control groups. No deleterious effects were noted. Although the toxicological profiles of the methyl and ethyl apocarotenoates are incomplete, the use of these substances seems unlikely to result in toxic effects or increased levels of vitamin A (55).

β-carotene β-Carotene is poorly absorbed from the gastrointestinal tract. In man, 30–90% of ingested β-carotene is excreted in the feces and absorption is not improved by concomitant fat intake. The major portion of absorbed β-carotene passes unaltered through the intestinal wall and reaches the liver via the portal vein, and to a much lesser extent via the lymphatics. Some β-carotene is stored in the liver and some is converted to vitamin A in the jejunum, liver, lung, muscle, and serum and stored in the liver as vitamin A (55, 58).

Hypercarotenemia is harmless and causes no symptoms, with the possible exception of carotenosis, a skin yellowing. No hypervitaminosis was noted in volunteers given β-carotene for extended periods. Beta carotene levels in the livers of two patients given β-carotene therapy for photosensitivity was found to be within normal range for subjects on a normal diet (77). Experimental hypervitaminosis A in animals induced by hypercarotenemia has been difficult to demonstrate because of poor intestinal absorption and conversion. No effects have been shown in rats given the equivalent of 40,000–70,000 IU of vitamin A esters/day intravenously, intraperitoneally, or by mouth. However, oral or subcutaneous doses equivalent to 1500 IU of vitamin A induced accelerated epithelial growth in rats and altered estrus (55). Acute oral toxicity of β-carotene in the dog was extremely low, $LD_{50} = 78$ g/kg (bw). A number of subchronic studies and a four-generation feeding study in rats at a dose of 1000 ppm in the diet for 2 years showed no adverse effects. A number of human subjects received 60 mg of β-carotene/day for 3 months; serum β-carotene levels doubled whereas vitamin A levels remained unchanged; no clinical effects were noted.

Annatto extract Little is known about the metabolic fate of annatto extracts (bixin, norbixin), although a number of toxicity studies have been conducted in experimental animals. Subchronic feeding studies in rats with 2% fat-soluble or 2% water-soluble annatto in the diet for 13 weeks resulted in no abnormalities. Beagle dogs received up to 20% water-soluble annatto extract in their diet for 16 weeks, followed by 36 weeks of 10% annatto in the diet and 10% in gelatin capsules. Growth inhibition and reduced food intake were observed at this high dose; however, mortality, liver and kidney function, hematology, and histopathology were normal. Life-span studies in 50 male and 50 female mice fed either 0.5% corn oil or 0.5% fat-soluble

annatto revealed no significant increase in tumor production. Groups of 10 male and 10 female rats received up to 0.5% fat-soluble or oil-soluble annatto for their life spans. The extracts contained 0.2–2.6% carotenoid as bixin. Two daughter generations were bred; each was fed similar diets for 7 and 8.5 months. No adverse effects were observed (55, 58).

Although it is well known that in rare susceptible persons some synthetic food colors can provoke hypersensitivity reactions such as urticaria, angioneurotic edema, and asthma, it is somewhat surprising to find similar reactions indicated for natural color exposure. Among 61 patients suffering from chronic urticaria and/or angioneurotic edema, 56 were orally provoked by annatto extract during elimination diet (78). In the study, 25 μl of a vegetable oil extract of annatto was used with a bixin (carotenoid) content of 0.065%. Some (26%) of the patients reacted to the annatto extract within 4 h of administration. Urticarial rashes were observed in 17 patients, whereas 7 exhibited angioedema. In this group of susceptible patients, similar challenges with synthetic colors gave the following incidence of reaction: tartrazine, 11%; sunset yellow, 17%; amaranth, 9%; erythrosine, 12%; Brilliant Blue FCF, 14%; and Ponceau 4R, 15%. There was no evidence of cross-reactivity between synthetic dyes and annatto extract. The active component of annatto extract is not known. Besides bixin and other carotenoids, the extract may contain numerous unidentified components.

The carotenoids of lesser importance, such as capsanthin (paprika extract) and crocin (saffron), are virtually unstudied from a biochemical and toxicological standpoint.

Betalaines

CHEMISTRY AND OCCURRENCE The red beet root (*Beta vulgarus*) contains red and yellow pigments of the class known as betalaines (25b, 34, 84). Red-violet betacyanins and yellow betaxanthines are water-soluble quaternary ammonium derivatives of 4-vinyl-5,6-dihydropyridine-2,6-dicarboxylic acid (Figure 2). Betanine (Table 3), the red pigment of the table beet, is probably the best known example of the betacyanins; its aglycone is betanidin.

STABILITY The stability of betanine was studied by Von Elbe et al (108). The red color of betanine remained unchanged between pH 4 and 7. Below pH 4 and above 7, the color shifts to red violet and violet, respectively, due to changes in ionization of acidic groups. The thermal stability of betanine is pH dependent and is greatest in the range of pH 4.0–5.0. Betanine in beet juice or certain other foods appeared to have enhanced thermal stability.

The chemical nature of thermal color loss is unknown and may be partially reversible (108). Other factors influencing betanine stability include air, light, radiation, water activity, metal ion contamination, and sequestrants. Despite the drawback of chemical or color instability, the betalaines possess high tinctorial strength comparable to many synthetic colorants and their stability exceeds that of the anthocyanins.

METABOLISM AND TOXICITY Relatively little data are extant on metabolism and toxicology of the betalaine alkaloids. Although they appear to be poorly absorbed, some 14% of the normal population experience beturia, a genetic error of metabolism in which the betalaines are excreted unchanged in the urine (86, 115). In a recent study with rats, only 3% of an oral dose of betanin was recovered in urine and 3% was recovered in the feces after 24 h. The remainder presumably was metabolized in the gut (62). Betanin is not pharmacologically inert and cardiovascular effects have been noted in man (62) and rats (115) after parenteral administration.

In a two-generation reproduction study in rats, 32 animals in the parental group received 17 g of betanine and produced 24 pups, which in turn received 25 g each over a mean life span of 800 days. Two mammary fibroadenomas were observed in the test group, but no other adverse effects (55).

Another group of 32 male and female rats received betanin in their drinking water to a total dose of 17 g/animal. No significant differences between the test group and 56 control animals were noted in mean life span or tumor incidences (55). No information is available on embryotoxicity, teratogenicity, or genetic toxicity.

Caramel

CHEMISTRY Caramel is a complex polysaccharide of unidentified chemical structure prepared by heating a food-grade carbohydrate such as glucose, sucrose, or starch in the presence of a catalyst—acetic, sulfurous, or citric acids or bases such as ammonium, calcium, or sodium hydroxides. Chemical composition varies with method of preparation. About 50% of the colorant is digestible carbohydrate, 25% is nondigestible carbohydrate, and 25% is melanodins (76, 103). Caramel can be considered a natural constituent of the diet since it is found when certain foods are cooked or when sucrose is heated. Toxicologically, there is no distinction between such naturally produced caramels and those produced commercially from food-grade carbohydrates, with the exception of caramels produced by the ammonia-ammonium sulfate process (55).

The induction of convulsions in cattle and sheep fed ammoniated sugar-containing feed supplements led to the discovery of pyrazines and imida-

zoles in the ammonia-treated molasses. The most likely toxic component was identified as 4-methyl imidazole, which elicits convulsant activity in rabbits, mice, and chicks. The pyrazines are mild CNS depressants and weak anticonvulsants. Methyl imidazole content of food-grade caramel was found to vary from 20–200 ppm, whereas commercial caramels contained up to 700 ppm. The imidazole content increases linearly with the molar ratio of ammonia to glucose.

METABOLISM In rats fed 30 ml of 10% and 20% caramel solutions, from starch hydrolysates with ammonium hydroxide and sulfurous acid catalysts, daily for 100 days, the average absorption was about 30% of the dose. Remaining caramel was extracted from the feces. Measured absorption would include degradation by intestinal microflora (103). Earlier studies revealed that intestinal enzymes cannot hydrolyze levoglucosan and that 5–19% of ingested levoglucoside appeared in the urine (42). Large amounts of ingested caramel lowered urinary nitrogen excretion (4), an effect attributed to larger quantities of fecal excretion. Large quantities of fermentable or partially fermentable carbohydrate, e.g. lactulose, glucose, mannitol, or sorbitol, in the colon are known to influence the pH of the lower bowel contents, inhibiting ammonia production and absorption (107).

TOXICITY A variety of caramel colors has been tested in subchronic feeding studies in rats and one caramel type was tested in dogs. The highest concentration employed were 20% (rat) and 25% (dog) of the diet. Aside from a mild reduction in growth rate at the higest dose in rats, no adverse effects were noted. A few long-term studies have also been conducted or are in progress, but little published information is available (55). In a teratologic evaluation of caramel in mice, rats, and rabbits, pregnant animals received 1.6 g/kg (bw)/day for 10–13 days. No significant effects were noted on nidation, on maternal or fetal survival, or on soft tissue or skeletal abnormalities between test and control groups. Mutagenic potential of caramel was evaluated in *Saccharomyces cerevisiae* D4 and *Salmonella typhimurium* (strains TA1535, TA1537, and TA1538), both with and without activation by mammalian liver homogenates. No mutagenicity was observed in these tests, but caramel exhibited high toxicity for the *Salmonella* strains and thus was tested only up to 350 ppm (71).

Curcumin (Turmeric)

Turmeric, a yellow-brown substance widely used as a spice and a natural colorant, is derived from the rhizome of *Curcuma longa* L.. It contains about 1–5% of curcumin (Table 3, Figure 2) as the principal colorant.

METABOLISM The metabolic fate of curcumin in the rat has been studied in relatively great detail. Orally administered in a dose of 1 g/kg (bw), 75% of it was excreted in the feces, with only trace amounts appearing in the urine (110). No toxic effects were apparent after doses of up to 5 g/kg (bw). In studies (50) that used ^{3}H-labeled curcumin somewhat less absorption occurred after an oral dose of about 2.2 g/kg (bw). Fecal and urinary excretion was 89 and 6%, respectively, after 72 h. After intraperitoneal dosage, 73% was excreted in the feces and 11% was excreted in the urine, indicating an efficient biliary excretion of curcumin. Most of the ^{3}H excretion occurred in the first 24 h. No significant amounts of radioactivity were found in the tissues 3 days after dosage. About 1% of the dose was found as ^3H_2O in urine and expired air. In bile duct-cannulated rats administered ^{3}H-labeled curcumin i.v., biliary excretion was very rapid, with 74% of dose excreted in 2 h. The nature of curcumin biliary metabolites was investigated by using mass spectroscopy. Major metabolites were glucuronides of tetrahydro- and hexahydrocurcumin; a minor metabolite was dihydroferulic acid. The reduction of the α,β-unsaturated bonds of curcumin is not unusual. Cinamic acids are reduced by the gut microflora (9, 92) and it seems likely that other α,β-unsaturated carbonyl compounds could also be reduced in the gut. Presumably, in this case, the metabolites are generated by an α,β-unsaturated ketone reductase. The origin of dihydroferulic acid is presumably via oxidative cleavage of reduced curcumin metabolites.

BIOLOGICAL EFFECTS AND TOXICITY Turmeric has been reported to exhibit a number of biological or medicinal properties, including anti-inflammatory (22), antiarthritic (22), and antibacterial (96) activity. Curcumin itself exhibits hypocholesteremic action in rats (85, 100) and antioxidant activity (97). No data are available on acute toxicity of curcumin; some subchronic and chronic studies have been conducted. Two dogs were fed for 1 year on a diet containing 1% commercial turmeric (~3% curcumin); no adverse effects were noted (55). Groups of 20 male and female rats were fed for 420 days on a diet containing 0.5% turmeric. Growth, hematology, and reproductive function were unaffected. No tumors were observed, although the period of exposure was only about half that now considered adequate, i.e. 24–30 months, for studies of this type. Data on reproduction, embryotoxicity, and teratogenicity are also inadequate (55). In studies (1) of chromosomal changes in root tip cells of *Allium cepa,* the predominant aberration induced by tumeric was chromosome breakage. Effects on spindle and cytokinesis were also indicated by the occurrence of *c*-mitosis, somatic segregations, binucleate cells, and multipolar anaphases. In another in vitro study that used cell strains of the Chinese hamster, cactus mouse, Indian muntjac, and short-term human lymphocyte

cultures, a number of cytogenetic effects were induced by turmeric (41). These included arrested mitosis, altered chromosome morphology, and altered nucleic acid synthesis. The metaphase chromosomes showed time- and dose-dependent uncoiling, chromatid separation, fragmentation, and disintegration. It is not known what component of turmeric was responsible for the cytogenetic effects observed. A recent study with either turmeric (0.5%) or curcumin (0.015%) in the diets of mice did not show significant increases in the incidences of micronucleated polychromatic erythrocytes, structural and numerical aberrations in bone marrow chromosomes, pregnancy rate, number of live and dead embryos, total implants, and mutagenic index (106). The same was true of rats fed cooked diets containing turmeric (0.5 and 0.05%). Curcumin is one of a number of agents tentatively selected for testing in the National Toxicology Program, Carcinogenesis Testing Program (81).

Miscellaneous Plant Pigments

CHLOROPHYLLIN COPPER COMPLEX, K AND Na SALTS Potassium sodium copper chlorophyll is a green to black powder obtained from chlorophyll by replacing the methyl and phytyl ester groups with alkali and the magnesium with copper. Chlorophyll is prepared from dehydrated alfalfa, and total copper content is 4–6%, with not more than 0.25% free ionic copper. Chlorophyllin copper complex, like chlorophyll, appears to be poorly absorbed from the gastrointestinal tract. No copper storage occurred in the liver, kidney, or spleen of rats fed up to 3.0% NaK chlorophyllin copper in the diet for 104 weeks (46). Reported increased plasma levels of copper were not associated with significant storage or destruction of ascorbic acid. Long-term feeding studies in rats at up to 3% copper chlorophyllin complex in the diet for their life span resulted in no adverse effects in growth rate, reproduction, histopathology, blood, or urine chemistry (46, 55). Other porphyrin pigments are discussed in a recent review (25b).

RIBOFLAVIN Riboflavin, a vitamin and food colorant, is essentially nontoxic. The oral LD_{50} in rats is greater than 10 g/kg (bw) and in dogs is greater than 2 g/kg (bw). The low solubility of riboflavin ($<$0.3 mg/ml of H_2O) limits absorption of massive doses. Daily administration for 4–5 months of 10 mg of riboflavin to rats and 25 mg/kg (bw) to dogs produced no adverse effects. Rats receiving 10 mg/day were bred through three generations (105). Although no chronic studies have been carried out in experimental animals, the low acute toxicity coupled with clinical and nutritional data on riboflavin (38) seem sufficient to justify its continued use.

SUMMARY

Evaluation of the chemical and biological data on synthetic and natural food colorants indicates that synthetic colorants in general have been studied more thoroughly than their natural counterparts. The increasing use of natural colorants in processed foods at levels higher than those found in natural sources has raised concern about deficiencies in knowledge, in some cases, of their exact chemical composition, stability, metabolic fate, and toxic potential. Additional studies, particularly of comparative metabolic fate in experimental animals and in man and of genotoxic potential, are required. Until studies with natural colorants comparable to those carried out with synthetic colorants have been completed and evaluated, greater safety of natural colorants cannot be assumed.

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