THE ROLE OF NATURAL COLOR ADDITIVES IN FOOD ALLERGY

CHRISTINE D. LUCAS, JOHN B. HALLAGAN

International Association of Color Manufacturers,
1620 I Street, NW, Suite 925
Washington, DC 20006, USA

STEVE L. TAYLOR

Food Processing Center
14E HC Filley Hall, East Campus
PO Box 830930
Lincoln NE 68583-0930, USA

I. INTRODUCTION

Interest in food allergy and intolerance has increased in recent years. As interest has increased, efforts to identify foods and food constituents that may cause reactions have also increased. A variety of foods and food constituents have been identified as causing reactions (Hefle *et al.*, 1996). The

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current consensus, as adopted by the Codex Alimentarius Commission of the World Health Organization, considers eight foods or food groups to be the major causes of food allergy (Codex, 1998). Natural color additives are justifiably not included among the foods and food groups identified by Codex.

"Natural" color additives are generally considered color additives derived from plant or animal sources by extraction or other physical processing. Examples include carmine/cochineal, annatto extract, grape skin extract, turmeric, saffron, and beta-carotene, which are the major natural color additives used in foods. "Synthetic" color additives include chemically synthesized substances and include tartrazine (FD&C Yellow No. 5), erythrosine (FD&C Red No. 3), and indigo carmine (FD&C Blue No. 2). While natural and synthetic color additives are subject to the same safety standards under the regulatory scheme delineated in the US Food, Drug, and Cosmetic Act, they are regulated in two classes by the US Food and Drug Administration (FDA) (Hallagan *et al.*, 1995). In general, the synthetic color additives are subject to a certification requirement to assure that each batch of material manufactured meets the mandated specifications while the natural colors are "exempt from certification" (see Table I) and may be manufactured and marketed without certification by FDA. Under the FDA regulatory scheme, there is no provision for designating a color additive as "natural." In fact, FDA's regulations forbid it unless the color additive is derived from the same food that it is being used to color. The appropriate terms in the US are "certified" color additives (synthetic), and color additives "exempt from certification" (natural).

TABLE I

COLOR ADDITIVES EXEMPT FROM CERTIFICATION IN THE USA (CFR CITATION)

Annatto (21 CFR 73.30)	Fruit juice (21 CFR 73.250)
Dehydrated beets (21 CFR 73.40)	Vegetable juice (21 CFR 73.260)
Ultramarine Blue (21 CFR 73.50)	Dried algae meal (21 CFR 73.275)
Canthaxanthin (21 CFR 73.75)	Tagetes (Aztec marigold) meal and extract (21 CFR 73.295)
Caramel (21 CFR 73.85)	Carrot oil (21 CFR 73.300)
β-Apo-8'-carotenal (21 CFR 73.90)	Corn endosperm oil (21 CFR 73.315)
β-Carotene (21 CFR 73.90)	Paprika (21 CFR 73.340)
Cochineal extract: carmine (21 CFR 73.100)	Paprika oleoresin (21 CFR 73.345)
Toasted partially defatted cooked cottonseed flour (21 CFR 73.140)	Riboflavin (21 CFR 73.450)
Ferrous gluconate (21 CFR 73.160)	Saffron (21 CFR 73.500)
Grape color extract (21 CFR 73.69)	Titanium dioxide (21 CFR 73.575)
Grape-skin extract (enocianina) (21 CFR 73.170)	Turmeric (21 CFR 73.600)
Synthetic iron oxide (21 CFR 73.200)	Turmeric oleoresin (21 CFR 73.615)

In the US, foods containing synthetic (certified) color additives must contain a declaration of the presence of these color additives on a specific and individual basis in the ingredient statement for the product. The presence of natural color additives (color additives exempt from certification) may be declared in a generic manner in the ingredient statement using a statement such as "artificially colored" or "color added"; no specific declaration is required. Some consumer groups have questioned the exemption status of "natural" color additives from specific label declaration, citing the possibility of adverse reactions. Based upon available data, the potential is very limited for natural color additives to cause allergic or intolerance reactions.

II. ADVERSE REACTIONS TO FOOD: ALLERGY AND INTOLERANCE

The differences between food allergy and intolerance are significant but poorly understood by the consuming public and even some individuals in the medical community. This situation often leads to confusion when describing adverse reactions to foods. Both food allergies and food intolerances involve abnormal responses to particular foods among certain sensitive individuals in the population. The same food is safe for the vast majority of consumers to ingest. However, food allergies can be distinguished from food intolerances on the basis of the mechanisms involved in the adverse reaction, the severity of the potential symptoms, and the degree of tolerance for the offending food.

True food allergies are abnormal reactions of the immune system to certain food components called allergens (Lemke and Taylor, 1994; Mekori, 1996). The allergens are typically naturally-occurring proteins in the specific food. Food allergies can be divided into two categories: immediate hypersensitivity and delayed hypersensitivity reactions. In immediate hypersensitivity reactions, symptoms begin to develop within a few minutes to a few hours after ingestion of the offending food. Immediate hypersensitivity reactions have been noted with many foods and can occasionally be severe and systemic (Taylor *et al.*, 1989). In delayed hypersensitivity reactions, symptoms do not begin to appear until 24 hours or longer after ingestion of the offending food (Taylor and Dormedy, 1998a). Only a few foods have been definitely linked to delayed hypersensitivity reactions, although further investigations concerning this possibility are clearly needed. The best known example of a delayed hypersensitivity reaction is celiac disease, which is an abnormal response to wheat, rye, barley, triticale, spelt, kamut, and possibly oats (Strober, 1986; Skerritt *et al.*, 1990). The symptoms involved in delayed hypersensitivity reactions are

likely to be more localized and less likely to be systemic. For example, in celiac disease, the adverse reaction is limited primarily to the small intestine. Since celiac disease does not involve colorants, it will not be discussed further. For both immediate and delayed hypersensitivities, the degree of tolerance for the offending food is quite low. Exposure to trace quantities of the offending food or food ingredient can elicit an adverse reaction (Hourihane *et al.*, 1997; Taylor *et al.*, 1999).

In contrast, food intolerances occur through a number of different non-immunologic mechanisms. The general categories of food intolerances include metabolic food disorders, anaphylactoid reactions, and idiosyncratic illnesses (Lemke and Taylor, 1994). Because of the variety of different mechanisms involved in food intolerances, a wide range of symptoms can be encountered in these illnesses. However, with a very few exceptions, these reactions are quite mild. Furthermore, food-intolerant individuals can often ingest small quantities of the offending food or food ingredient without provoking adverse reactions (Taylor, 1990).

A. FOOD ALLERGY

The course of the immune responses to an ingested food is a series of complex and integrated steps determined by many different factors, including the nature and type of the proteins present in the food which can act as antigens or allergens stimulating the immune system (Mekori, 1996; Sampson, 1999). Ingestion of proteins with foods can result in stimulation of the immune system especially if the proteins are somewhat resistant to digestion. Antigens are proteins that can stimulate any type of immunological response. The typical responses involve stimulating the production of antigen-specific IgM, IgG, or IgA antibodies. These sorts of immune responses are generally harmless. Allergens are proteins that can stimulate an allergen-specific IgE response. Humans ingest literally millions of different proteins with their foods. Oral tolerance develops to most of these proteins (Sampson, 1999); oral tolerance is associated with the production of IgG, IgM, and IgA antibodies. However, in an estimated 2–6% of adults and children (Taylor *et al.*, 1999), a rather small number of ingested proteins are able to act as allergens and stimulate abnormal hypersensitivity reactions (Bush and Hefle, 1996).

1. Immediate Hypersensitivity

Immediate hypersensitivity reactions are mediated by a specific class of antibodies known as immunoglobulin E or IgE (Mekori, 1996). The development of an IgE-mediated response to a specific protein is the result of a

complex series of events in the immune system involving antigen-presenting cells, T cells, and B cells (Sampson, 1999). All humans have low levels of IgE antibodies, but only individuals with the predisposition to develop allergies will produce IgE antibodies that are specific for and recognize certain protein allergens from food or the environment. While the allergens are typically proteins, only a few of the many proteins in nature are capable of stimulating the production of specific IgE antibodies in susceptible people. Such allergens can be found in pollens, mold spores, bee venoms, animal danders, dust mites, and foods. In the sensitization phase of the immediate hypersensitivity reaction, exposure to the allergen stimulates the production of allergen-specific IgE antibodies by the B cell. These antibodies are released from the B cells and attach to the membranes of mast cells in various tissues and basophils in the blood. Although the IgE antibodies are formed during the sensitization phase, no allergic reaction occurs until after the IgE antibodies become affixed to the mast cells and basophils. Upon subsequent exposure to the allergen, the allergen cross-links two IgE antibodies on the surface of the mast cell or basophil membrane. This stimulates the release of a host of potent chemical mediators of the allergic response into the tissues and blood. Although many mediators have been described, histamine is one of the primary mediators. Histamine is responsible for many of the most immediate symptoms that are noted in IgE-mediated allergic reactions. The typical symptoms of allergic reactions to foods can involve the gastrointestinal tract (nausea, vomiting, diarrhea), the skin (hives, eczema, itching, swelling), and the respiratory tract (asthma, rhinitis, laryngeal edema or throat swelling). In unusual cases, ingestion of specific foods can trigger anaphylactic shock in highly sensitive individuals. Anaphylactic shock involves symptoms occurring simultaneously in all the organ systems noted above along with cardiovascular manifestations including a profound drop in blood pressure (hypotension). Death can occur from anaphylactic shock within minutes of ingestion of the offending food if appropriate treatment is not received. The mechanism of IgE-mediated, immediate hypersensitivity reactions is depicted in Fig. 1.

2. *Delayed Hypersensitivity*

In contrast, delayed hypersensitivity reactions do not involve formation of allergen-specific antibodies and take 24–72 hours to develop (Sampson, 1990). Instead, delayed food hypersensitivity reactions are governed by cytokine-secreting, sensitized T cells which contain allergen-specific cell membrane receptors (Ring and Thewes, 1999). Upon exposure to the allergen, the T cells secrete lymphokines that induce tissue inflammation by

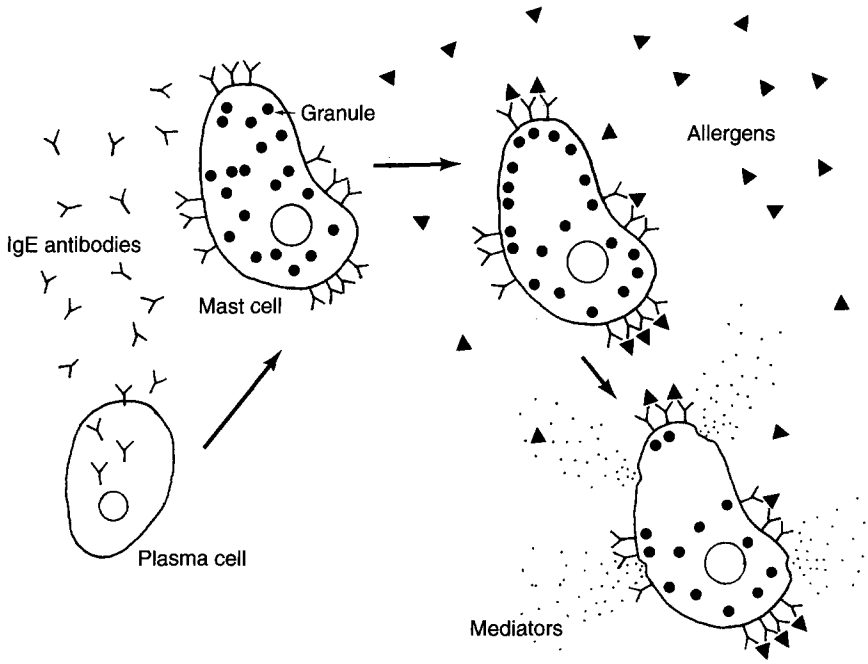

FIG. 1. Mechanism of IgE-mediated reaction.

attracting phagocytic cells. Activated phagocytic cells release lytic enzymes which can destroy surrounding tissue thereby eliciting an allergic response (Taylor and Dormedy, 1998a). In contrast to antibody-mediated reactions, allergic manifestations of cell-mediated allergies are more likely to be localized and do not involve systemic reactions, e.g. anaphylactic shock (Lemke and Taylor, 1994). The precise role of cell-mediated immune reactions in food allergies is unknown with the exception of celiac disease which was described earlier.

Contact sensitivity is a cell-mediated and delayed cutaneous reaction which develops after repeated and prolonged contact with an allergen. The majority of contact sensitivity reactions are controlled by the T cells or Langerhan cells of the dermis or epidermis, which contain specific regions on their surfaces to bind antigens or haptens (Ring and Thewes, 1999). These haptens are usually low molecular weight chemicals that are reactive with proteins. Haptens are reported to be the main causative agent in contact sensitivity reactions (Taylor and Dormedy, 1998a). These haptens would not be antigenic by themselves but are able to penetrate the epidermis and become conjugated to normal body proteins. Langerhans cells

have the capacity to bind these small molecular weight compounds and to present the hapten/protein conjugate to the major histocompatibility complex resulting in the proliferation of sensitized T cells (Ring and Thewes, 1999). Contact hypersensitivity is the result of sensitized T cells infiltrating the epidermis, recognizing the hapten/protein conjugate, and causing inflammatory changes through the release of specific cytokines from the T cells, Langerhans cells, and keratinocytes (Ring and Thewes, 1999).

B. FOOD INTOLERANCES

Food intolerances occur through a variety of non-immunological mechanisms. As noted earlier, food intolerances can be divided into three classes including anaphylactoid reactions, metabolic disorders, and idiosyncratic reactions (Lemke and Taylor, 1994).

Similar to an IgE-mediated allergic response, anaphylactoid reactions involve the release of histamine and other mediators from mast cells and basophils, but, in this case, allergen-specific IgE antibodies are not involved. Theoretically, certain substances in foods destabilize the membranes of the mast cells and basophils causing the release of histamine and other mediators. However, none of these histamine-releasing agents has ever been identified in foods. Thus, the evidence for the existence of this mechanism in food intolerances is mostly circumstantial.

Metabolic disorders are generally enzyme deficiencies that result in an inability to digest a particular food or food ingredient or result in a heightened sensitivity to the substance (Lemke and Taylor, 1994). Metabolic food disorders often involve inherited traits. A good example is lactose intolerance that results from an inherited deficiency of the enzyme, β-galactosidase, in the small intestine (Kocian, 1988). Without this enzyme, lactose cannot be digested and used for metabolic energy. Instead, lactose is transported into the colon where it is fermented by intestinal bacteria causing flatulence and frothy diarrhea.

Idiosyncratic reactions refer to that category of intolerance reactions for which the mechanism of action is unknown (Lemke and Taylor, 1994). Unexplained adverse reactions to food additives are commonly characterized as food idiosyncrasies. In many cases, the cause-and-effect relationship between the specific food additive and the adverse reaction remains to be clearly established. However, in a few cases, the cause-and-effect relationship is well established. An example would be sulfite-induced asthma (Taylor *et al.*, 1997). Sulfites trigger asthmatic reactions in a rather small segment of the asthmatic population, but the mechanism of action for sulfites remains elusive.

III. REACTIONS TO NATURAL COLOR ADDITIVES

The coloring compounds in natural color additives are small molecular weight, non-protein chemicals that would not be expected to elicit true food allergies, either IgE-mediated or cell-mediated. These compounds could only stimulate the immune system by binding to proteins and acting as haptens. No evidence exists to suggest that any of the natural color additives act as a hapten to provoke allergic reactions. However, natural color additives are often extracts of biological materials that may contain many other components, including proteins in addition to the coloring compounds. Reactions to "natural" color additives are reported occasionally and are attributed to the presence of protein residues in colors such as carmine and annatto (Taylor and Dormedy, 1998b). The levels of protein residues present in carmine and annatto may vary depending upon processing. Some types of processing of these color additives and the food containing them would be expected to eliminate the protein residues or render them non-allergenic in the final food product. Although the mechanisms involved in these reported cases of adverse reactions to natural color additives are often not investigated, many of these reports probably involve IgE-mediated reactions with the IgE antibodies being directed against the protein residues remaining in the coloring formulation. No evidence exists to suggest the involvement of natural color additives in cell-mediated allergic reactions or in any of the various types of food intolerances. The discussion here is limited to case reports of reactions following ingestion of natural color additives and/or studies using oral administration. Since these same substances are also used in cosmetics, reports of contact sensitivities also exist but will not be reviewed here. A few reports exist of occupational allergies from either the inhalation of or contact with natural color additives but these reports will be discussed in this review only in relation to the sensitivity of these same individuals to ingested natural colors.

A. CARMINE

Carmine is a dark red color additive obtained by aqueous extraction of cochineal, which is derived from the dried bodies of the gravid female insect *Coccus cacti*. Carmine consists of an aluminum or calcium-aluminum lake on an aluminum hydroxide substrate of carminic acid, the coloring principle of cochineal (Marmion, 1991). Carminic acid is an hydroxyanthraquinone linked to a glucose unit and it comprises approximately 10% of cochineal and 2–4% of its extract (Marmion, 1991). Commercial preparations of carmine contain 20–50% carminic acid (Madsen *et al.*, 1993).

Reports of Reactions to Carmine

Although carmine is widely consumed in foods and beverages, it has been rarely implicated in adverse reactions experienced by consumers. The typical low levels of carmine in foods and beverages limits oral exposure to this color additive. Thus, the likelihood of sensitization to carmine by ingestion is rather low. The most probable mechanism involved in adverse reactions to carmine is an IgE-mediated allergy. The sensitization would occur to protein residues present in carmine (Acero *et al.*, 1998), and the carmine-specific IgE antibodies would be directed against one or more of those specific carmine-associated proteins. However, the protein content of carmine is likely quite small. Thus, the low level of exposure to carmine-associated allergenic proteins makes allergic sensitization unlikely among consumers of carmine-associated foods and beverages. IgE sensitization to carmine-associated allergens is more likely to occur through higher level exposures such as occupational or cosmetic exposure to carmine. Once IgE sensitization to these carmine proteins occurs, the level of exposure to these residual proteins through carmine-containing foods and beverages may be sufficient to elicit allergic reactions. The proteinaceous allergens in carmine have yet to be identified. Also, the stability of these allergens to food processing conditions, including their heat stability, has not been investigated. However, the allergenicity of carmine may be affected by food processing conditions, especially if the allergens are unstable to heat.

In one of the first reports, 24 atopic and non-atopic patients diagnosed with irritable bowel syndrome maintained an exclusion diet which consisted of lake fish, rice, dried apricots, bread made of corn and soya, and mineral water for three weeks. Following the three-week period, other foods were reintroduced into the diet and removed again if suspected of causing an adverse reaction. One non-atopic patient showed improvement upon removal of carminic acid, but this result could not be confirmed by blind challenge or skin prick test (Petitpierre *et al.*, 1985).

In a more recent investigation, an anaphylactic reaction was reported in a 34-year-old female atopic patient after ingestion of a Campari-Orange® beverage, which contains carmine (Kagi *et al.*, 1994). Her symptoms included widespread urticaria (hives), rhinitis, nausea, vomiting, bronchospasm (asthma or wheezing), chills and diarrhea. Skin prick tests to the Campari-Orange® beverage, carmine, and cosmetics containing the dye were positive and were considered an indication of carmine- or cochineal-specific IgE. The radioallergosorbent test (RAST) was performed to detect IgE antibodies in the patient's blood serum. An initial RAST test was negative, but after a year, the test was positive for circulating IgE antibodies specific

to carmine. The patient reported experiencing itching skin and burning eyes following the application of eyeshadow, blush or lipsticks; the authors did not identify the brand. The authors reported that sensitization to carmine was probably due to the use of carmine-containing cosmetics.

Beaudouin *et al.* (1995) described a reaction to carmine in a 35-year-old woman after she ingested yogurt that contained mixed fruits. Approximately two hours after the patient consumed the yogurt, she experienced symptoms of anaphylaxis including generalized urticaria, angioedema (localized swelling), and asthma. The patient reported similar allergic reactions previously after eating certain foods, including delicatessen meats, chocolate, and yogurt with fruit. Six weeks after the anaphylactic episode, skin prick tests were performed using the yogurt, which she had consumed the day of her reaction, and carmine; both skin prick tests were positive. A leukocyte histamine release test was performed using the patient's blood basophils to determine if exposure of these cells to carmine would elicit the release of histamine. This test was determined to be positive. The investigators estimated that the patient ingested approximately 1.3 mg of carmine. However, this estimate of intake should be questioned because a serving of yogurt would more likely contain approximately 25 mg of carmine. This individual appeared to have an IgE-mediated reaction to carmine based upon the positive skin prick test and the histamine release assay.

More recently, four additional cases of adverse reactions following consumption of alcoholic beverages containing carmine were reported in women ranging from 25 to 43 years old (Wüthrich *et al.*, 1997a). In the first case, a 33-year-old atopic woman reported experiencing urticaria and angioedema several times following meals and twice after consuming Campari-Orange®. Skin prick tests were performed and were positive for carmine supplied by Campari® (1:1 dilution) and weakly positive for commercially available carmine (0.5% carmine). Skin prick tests with carmine can be compromised on occasion by the solubility of carmine proteins. The RAST for carmine (prepared using carmine supplied by Campari®) was positive. The second case reported was a 43-year-old woman who on two separate occasions experienced rhinorrhea (profuse nasal discharge), eyelid edema (swelling), pruritus (itching), and dyspnea (shortness of breath) after drinking Campari-Bitter® or Campari-Orange®. The skin prick tests were positive for carmine supplied by Campari® and negative for commercially available carmine, perhaps owing to the solubility problem noted above. In the third case, an atopic 25-year-old woman reported sneezing, rhinitis, nasal obstruction, angioedema, widespread urticaria and dyspnea within 30 minutes of consumption of Campari-Orange® beverage. Skin prick tests were positive with Campari® and carmine supplied by

Campari®, but negative with commercially available carmine. The RAST for carmine was positive. In the fourth case, after drinking Campari-Orange®, a 39-year-old woman developed acute urticaria with angioedema of the face within 30 minutes. After 30 minutes, skin prick tests performed using Campari® carmine were positive. Skin prick tests using commercially available carmine were negative. The RAST for carmine was positive.

In a recent case report, an anaphylactic reaction was reported in a 27-year-old woman after the consumption of a popsicle colored with carmine (Baldwin *et al.*, 1997). Immediately following ingestion of the popsicle, the patient experienced nausea. Within three hours, her symptoms included pruritus, urticaria, and hypotension (drop in blood pressure) with tachycardia (rapid heart beat). The patient recovered after hospitalization and treatment with epinephrine, intravenous fluids, and diphenhydramine (an antihistamine). The patient reported a previous reaction to carmine immediately following the application of a carmine-containing blush directly to facial skin. Several weeks following the initial anaphylactic episode, skin prick tests were performed using the popsicle and carmine on the patient and her husband, in addition to 20 control subjects who underwent skin prick testing to carmine. Skin prick tests and/or open oral challenges were also performed on the patient using other ingredients reported to be present in the popsicle or ingredients within other processed foods. The patient's skin prick test was positive to carmine and the popsicle and negative to the other tested materials; the open oral challenge to ingredients other than carmine found in the popsicle were negative. All 20 control subjects' and the patient's husband's skin prick tests were negative to carmine.

As further proof of the involvement of an IgE mechanism for this reaction, the Prausnitz-Kustner (P-K) test was performed using the patient's husband as the recipient. In the P-K test, blood serum, presumably containing allergen-specific IgE, is taken from the patient. An aliquot of the serum is heated to destroy the IgE, while another aliquot is left unheated. The heat-treated and the unheated serum from the patient is injected intradermally into sites on the recipient's arm. Later, skin prick tests are performed to determine if IgE from the patient can be passively transferred to the recipient's arm. This test is considered to provide very strong proof for the existence of IgE antibodies specific for the allergen. In this particular case, skin prick tests to carmine and the popsicle repeated at the serum-injected sites on the husband's arm conducted 63 hours after passive transfer were positive on the arm injected with unheated serum and negative on the arm injected with heated serum. The authors cited the positive results of the skin prick test to both the popsicle and carmine, negative skin prick tests and negative open oral challenges to the other popsicle

ingredients, and the patient's husband's positive skin prick to carmine following the P-K test as evidence of an IgE-mediated mechanism for the patient's anaphylaxis.

Kume *et al.* (1997) described four instances of acute allergic reactions in a 28-year-old female patient after ingestion of cochineal-containing beverages, specifically Campari®, strawberry milk and red-colored cocktail. Her symptoms included widespread urticaria, abdominal pain, fever, throat discomfort and diarrhea. After her most recent adverse reaction (time unspecified), the patient was administered a "small volume" (amount unspecified) of Campari® beverage and developed within two hours the following: pruritius, angioedema of the eyes, severe diarrhea and abdominal pain. The patient's skin prick tests to the Campari® beverage, strawberry milk beverage and cochineal color were positive indicating the involvement of IgE antibody in this reaction.

Occasionally, occupational exposure can result in sensitization to ingested carmine. Burge *et al.* (1979) identified two individuals with carmine-associated occupational asthma who reacted to oral challenges with carmine solutions. The first individual was employed at a dye manufacturing facility and experienced asthma and gastrointestinal upset after oral challenge with 1 ml of cochineal diluted in 100 ml of water. The second was employed at a cosmetic manufacturing operation and experienced asthma after oral challenge with Campari®.

In another such case, a 35-year-old non-atopic male patient employed at a spice warehouse and presenting with carmine-associated occupational asthma reacted positively to a double-blind, placebo-controlled oral challenge test with carmine at dose levels up to 150 mg (Acero *et al.*, 1998). Skin prick tests to carmine and cochineal extract were also positive. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate proteins from carmine and cochineal extract. When stained for protein, the SDS-PAGE gel revealed more protein bands and better electrophoretic separation with cochineal insect extracts as opposed to carmine. Based upon these results, the authors used cochineal insect extracts instead of carmine for immunoblotting. Immunoblotting results revealed specific IgE antibodies in the serum of this individual for high molecular weight proteins from the cochineal extract. Immunoblotting involves incubating the SDS-PAGE gel with human serum from the allergic individual. Any allergen-specific IgE in the serum will bind to the allergenic protein(s) on the gel. This binding can be documented by using some type of labeled (radioactive, enzyme-linked, etc.) antiserum against human IgE.

In contrast, an open oral challenge to an aqueous solution of 100 mg carmine failed to elicit any adverse reactions in another patient with carmine-associated occupational asthma, although skin prick tests were

positive (Quirce *et al.*, 1994). Thus, individuals with carmine-associated occupational asthma may be at some risk on ingestion of carmine-containing foods, although this does not always occur.

B. ANNATTO

The annatto tree (*Bixa orellana*) is a large, fast-growing shrub cultivated in tropical climates. The tree produces large clusters of brown or crimson capsular fruit-containing seeds coated with a thin, highly colored resinous coating that serves as the raw material for the preparation of the colorant annatto. Annatto is prepared by leaching the annatto seeds with an extractant prepared from one or more food-grade materials taken from a list that includes various solvents, edible vegetable oils and fats, and alkaline aqueous and alcoholic solutions (Marmion, 1991). The chief coloring principle found in the oil-soluble extracts of annatto is the carotenoid bixin (Marmion, 1991). The major colorant in alkaline aqueous extracts of annatto is norbixin (Marmion, 1991).

Reports of Reactions to Annatto

Twenty-five μl of annatto extract in gelatin capsules was administered to 56 patients who had previously suffered from chronic urticaria and angioedema. While 27% of the patients revealed symptoms of urticaria and angioedema after ingesting annatto (Mikkelson *et al.*, 1978), this study is flawed. Some patients were not symptom-free when the annatto challenge was administered, and the study design did not include placebo controls or double-blind challenges. Additionally, drugs controlling the chronic urticaria or angioedema may have been withheld from these subjects prior to challenge. The withholding of such drugs along with the failure to use placebo controls means that the responses could have been caused either by the additive or as a result of breakthrough urticaria from drug withdrawal. Because of the poor controls used in this study and the use of chronically ill patients, the results are not interpretable.

In another study, 112 patients suffering from angioedema and recurrent urticaria were administered 5 and 10 mg of annatto dye in open oral challenges during provocation tests to a variety of substances. Of the patients, 10% had a positive challenge while 14% had uncertain results (Juhlin, 1981). Again, the use of patients with chronic, recurrent urticaria in clinical trials can lead to difficulties in interpretation. As in the previous study, the failure to use placebo controls and the withdrawal of the patients from critical medications could have resulted in false positive reactions. This study is not conclusive.

In a more convincing case, an anaphylactic reaction was reported for a 62-year-old male after ingestion of Fiber One™ cereal which contains wheat bran, corn bran, aspartame, corn syrup, vitamins A, C, D, B_6, B_{12}, thiamine, and annatto extract color (Nish *et al.*, 1991). Within minutes, the patient developed symptoms characteristic of anaphylactic shock, including generalized pruritius, generalized urticaria, angioedema of the eyes and lips, undetectable blood pressure, and loss of consciousness. Skin prick tests to milk, corn, wheat, and annatto (full-strength, 1:1000 and 1:10,000 dilutions) were conducted five weeks after the incident. The skin prick tests to milk, corn, and wheat were negative. For annatto, the skin prick test at the 1:10,000 dilution was negative while the 1:1000 dilution and full-strength tests were positive. The patient's serum was positive for the presence of an annatto specific IgE when analyzed by immunoblot. This is the first report of anaphylactic shock due to the ingestion of annatto.

A possible asthmatic reaction associated with the ingestion of a pharmaceutical product containing annatto was described in a 53-year-old female (van Assendelft, 1984). However, no challenge tests or skin testing were conducted to confirm the physician's speculation that this patient was allergic to annatto.

In a recent, large clinical study of the prevalence of adverse reactions to food additives, 271 (98 controls and 173 with atopic symptoms) children underwent open oral challenges to the following food additives which were prepared in a lemonade solution: preservatives, natural colorings, synthetic colorings, flavorings and acids. Seventeen children had positive reactions to the open oral challenges. Following the open oral challenges, 12 of the 17 children with positive open oral challenges underwent double-blind placebo-controlled oral challenges test to the food additives which were enclosed in gelatin capsules. Of the 12, 5 reacted positively to the synthetic food colorings and one reacted positively to citric acid. None reacted positively to the natural colorings capsule which contained a mixture of 2.5 mg turmeric/100 ml and 1.6 mg annatto/100 ml, 6.0 mg β-carotene/100 ml, 1.0 mg canthaxanthin/100 ml and 5.5 mg beet coloring/100 ml (Fuglsang *et al.*, 1993).

In an extension of this investigation, 335 children underwent open oral challenges to the following food additives: preservatives, mixture of natural colorings and synthetic colorings, flavorings and acids. Twenty-three children had positive reactions to the open oral challenges. Following the open oral challenges, double-blind placebo-controlled oral challenges were conducted at the patients' homes with each group of additives. The natural colorings capsule contained a mixture of colorants as described in the preceding paragraph (Fuglsang *et al.*, 1993). Of the 16 patients participating in

the double-blind, placebo-controlled challenge, two patients reacted positively following consumption of the natural colorings capsule. The first patient experienced atopic dermatitis, while the second patient reported symptoms of urticaria (Fuglsang *et al.*, 1994). Because a mixture of colorants was employed in the capsule challenge, the role of annatto in the two positive reactions remains unclear.

In another similar challenge study of the prevalence of intolerance to food additives, 132 subjects underwent high and low dose challenges with various food additives; 81 children completed the study (Young *et al.*, 1987). The study involved administration of gelatin capsules containing a combination of food additives, including annatto at a minimum dose level of 1 mg and a maximum dose level of 10 mg. The challenges were conducted in double-blind, placebo-controlled fashion. The authors reported that the prevalence of reactions to annatto in the population was estimated to be 0.01 (lower limit) and 0.07 (upper limit) with a 95% confidence interval.

A randomized, double-blind, placebo-controlled oral food challenge was conducted in a group of 101 patients (25 male/76 female) suffering from eczema (Veien *et al.*, 1987). Following a standard elimination diet, the patients were orally challenged with five different capsules, containing the following food additives: sodium benzoate, sodium propionate, sorbic acid, 90 mg mixture of food colorings (including 10 mg annatto extract, 10 mg erythrosine, 10 mg ponceau 4R, 10 mg tartrazine, 10 mg patent blue V, 10 mg sunset yellow, 10 mg betanine, 10 mg curcumin and 10 mg quinoline), and placebo. Twenty-five patients reacted to the mixture of food colorings, while 76 did not. Sixteen patients reacted to the placebo. The number of reactions to the capsule containing the food coloring mixture was not statistically significant when compared to the number of reactions elicited by the placebo. The reactions seen in the first challenge could only be reproduced in a second challenge in one third of the patients. This study failed to yield conclusive results.

C. TURMERIC/CURCUMIN

The yellow color additive turmeric is the ground powder of the rhizomes of the *Curcuma longa* Linnaeus plant. Turmeric contains 3–5% volatile oils and 2.5–6% yellow pigments, the curcuminoids, of which curcumin predominates. The oleoresin is prepared via extraction from turmeric with one of the approved organic solvents. Following the evaporation of the solvent, turmeric oleoresin may contain 15–40% curcuminoids, along with volatile oils and other extractable plant constituents (Marmion, 1991).

Reports of Reactions to Turmeric/Curcumin

Turmeric pigments have been included in mixtures of natural colorings (2.5 mg turmeric/100 ml) administered during double-blind, placebo-controlled food challenges and are described above (Fuglsang *et al.*, 1993, 1994). In one study (Fuglsang *et al.*, 1993), the challenges were negative to mixtures of natural food colorings while in the second study, two positives were reported. The first patient experienced atopic dermatitis (eczema), while the second patient reported symptoms of urticaria (Fuglsang *et al.*, 1994). But, it is not possible to determine which of the natural food colorants may have triggered this adverse reaction. In a separate double-blind, placebo-controlled oral food challenge (described above) (Veien *et al.*, 1987) patients were orally challenged with a capsule containing a mixture of food colorings, including 11% curcumin. The number of reactions to the capsule containing the food coloring mixture was not statistically significant when compared to the number of reactions elicited by the placebo. The reactions seen in the first challenge could only be reproduced in a second challenge in one third of the patients (Veien *et al.*, 1987). Thus, no convincing evidence exists of allergic reactions to turmeric/curcumin.

D. CAROTENOIDS – β-CAROTENE AND CANTHAXANTHIN

Beta-carotene is an isomer of the naturally occurring carotenoid, carotene. Carotene is the pigment largely responsible for the color of various products obtained from nature, including butter, cheese, carrots, alfalfa, and certain cereal grains. The colorant is synthetically produced from acetone, which results in the formation of the all-*trans* form. Both the synthetically produced and the natural sources of β-carotene can be used as color additives (Marmion, 1991).

Canthaxanthin was isolated in 1950 from an edible mushroom (*Cantharellus cinnabarinus*), and has since been isolated from sea trout, algae, daphnia, salmon, brine shrimp, and several species of flamingo. Canthaxanthin may be prepared synthetically from acetone or β-ionone using procedures similar to those used for β-carotene (Marmion, 1991).

Reports of Reactions to Carotenoids

Reported cases of adverse reactions to natural colors belonging to the carotenoid color family are rare. Carotenoid colors have been included in mixtures of natural colorings (6.0 mg β-carotene/100 ml, 1.0 mg canthaxanthin/100 ml) administered during double-blind, placebo-controlled food challenges and are described above (Fuglsang *et al.*, 1993, 1994). In the

earlier study (Fuglsang *et al.*, 1993), the challenges were negative to the mixtures of natural food colorings, while in the later study two positives were reported. The first patient experienced atopic dermatitis, while the second patient reported symptoms of urticaria (Fuglsang *et al.*, 1994). Again, because of the use of a mixture of colorants, these reactions may not have been caused by carotenoids.

In another study previously described, 112 patients suffering from angioedema and recurrent urticaria were orally administered 50 and 100 mg β-carotene and 10 and 200 mg canthaxanthin in open fashion during provocation tests to a variety of substances. Of the patients 10% had a positive challenge to β-carotene, while 14% had uncertain results; 14% of the patients reacted positively to canthaxanthin, while 24% had uncertain results (Juhlin, 1981). As noted earlier, the design of this clinical study was flawed and the results are questionable.

Only one case report of a possible reaction to carotenoid colors was found in the literature (Greenbaum, 1979). An adverse reaction to vitamin drops and various foods was reported in a nine-month-old male infant. The patient's symptoms included atopic dermatitis, vomiting and colic, and restlessness. The authors confirmed sensitivity to vitamin A drops with double-blind challenges. Skin tests were negative. The authors suggested that the source of sensitivity was vitamin A and possibly carotenoid pigments. However, the suspected causative role for carotenoids in this case report was not conclusively established.

E. SAFFRON

Saffron color comes from the crocus plant. Each blossom of the crocus plant contains one pistil, consisting of three stigma, a style, and an ovary (Farrell, 1985). The saffron spice consists of the dried stigmas and style of the crocus bulb. The saffron coloring matter, crocin, also comes from the dried stigmas and style (Farrell, 1985). Crocin is easily hydrolyzed to crocetin and *d*-glucose *in vivo* (Farrell, 1985).

Reports of Reactions to Saffron

Anaphylactic reactions to apples, nuts and spinach, and asthma after ingestion of a meal of saffron rice and mushrooms were reported in a 21-year-old atopic farmer with mild atopic dermatitis oral allergy syndrome (hives, angioedema, and itching confined to the face, mouth, and throat area) (Wuthrich *et al.*, 1997b). Skin prick tests were performed using rice, saffron, mushrooms, garlic and onion. All were negative with the exception of saffron which gave a strongly positive result. A RAST test was

performed to detect IgE antibodies to saffron extract in the patient's blood serum. The RAST test was positive for both the retail saffron and a pure preparation supplied by a saffron manufacturer. SDS-PAGE followed by immunoblotting revealed specific IgE antibodies for proteins with molecular weights between 40 and 90 kDa.

F. GRAPE ANTHOCYANINS – GRAPE SKIN EXTRACT OR GRAPE COLOR EXTRACT (ENOCIANINA)

Anthocyanins are widely distributed in the plant kingdom where they occur as glycosides (i.e. associated with a sugar moiety) in combinations that produce red, blue or purple coloration in a variety of fruits and vegetables. Grape color extract and grape skin extract (enocianina) are anthocyanin-containing color additives approved for use in the United States.

Grape color extract is an aqueous solution of grape anthocyanin pigments made from Concord grapes or a dehydrated water-soluble powder prepared from the aqueous solution. The aqueous solution is prepared by extracting the pigments from precipitated lees produced during the storage of Concord grape juice. It contains the common components of grape juice, namely anthocyanins, tartrates, malates, sugars, and minerals but again not in the same proportion as found in grape juice. Water-soluble pigments such as 3-mono- and 3,5-di-glucosides of malvidin, delphinidin and cyanidin, and their acylated derivatives, are responsible for the purple color of grape color extract (Marmion, 1991).

Grape skin extract is a purplish-red liquid prepared by the aqueous extraction (steeping) of the fresh deseeded marc remaining after grapes have been pressed to produce grape juice or wine. It also contains the common components of grape juice, namely, anthocyanins, tartric acid, tannins, sugars, minerals, etc., but not in the same proportions as found in grape juice. The properties of grape skin extract are similar to those of grape color extract (Marmion, 1991).

Reports of Reactions to Grape Anthocyanins

Numerous adverse reactions, sensitivities and confirmed allergic reactions following ingestion of grapes or grape products have been reported in the literature (Eyermann, 1935; Kahn, 1942; Tuft and Blumstein, 1942; Eriksson *et al.*, 1982; David, 1984; Eriksson, 1984; Frankland and Aalberse, 1987; Kivity *et al.*, 1988; Ortolani *et al.*,1988; Moyer, 1990; Dohi *et al.*, 1991; Esteve *et al.*, 1993; Parker *et al.*, 1993; Steinman and Potter, 1994; Garcia-Ortiz *et al.*, 1995; Fernandez-Rivas *et al.*, 1997; Vaswani *et al.*, 1998). No reactions have been reported in the literature to either grape skin

extract or grape color extract. The allergic reactions reported to grapes are likely from exposure to protein in the grapes that would not be present in either grape skin extract or grape color extract.

IV. SUMMARY AND CONCLUSIONS

A critical evaluation of the available information demonstrates that reactions to natural color additives are rare. Studies of turmeric and carotenoid pigments administered in mixtures with other food colorings failed to definitely identify reactions to either color additive. For carotenoids, the one case report of an adverse reaction was not conclusive. An anaphylactic reaction to saffron does suggest an IgE-mediated reaction, but the high use of saffron as compared with this single report of an adverse reaction suggests that sensitivity to saffron is extremely rare. Numerous reports of reactions to grapes or grape products have been reported in the literature, but no reports of sensitivities to grape skin extract or grape color extract were found.

In rare cases, annatto dye may provoke a severe, adverse reaction in individuals with an uncommon hypersensitivity, and may aggravate the symptoms of patients suffering from recurrent urticaria. In its long history of use, there has been only one reported case of anaphylaxis resulting from the ingestion of annatto. Studies designed to investigate the role of annatto in recurrent urticaria sufferers were limited due to the absence of double-blind challenge and placebo controls.

A number of cases of adverse reactions to carmine following ingestion have been reported in the literature. These adverse reactions suggest an IgE-mediated hypersensitivity. In many of the reported cases, the cause of sensitization to carmine was topical exposure from the use of carmine-containing cosmetics or occupational exposure to carmine and not from ingestion of carmine-containing foods and beverages. Following sensitization, affected individuals would be sensitive to carmine and the amounts present in foods and beverages could elicit allergic reactions. It is not known whether all individuals with carmine sensitivity induced through topical use are sensitive to the ingestion of carmine in foods. However, reactions to carmine solely because of ingestion are likely to be exceedingly rare due to the low use levels of carmine in foods and beverages.

Despite their widespread use in food products, few reports of allergic reactions following ingestion have been reported for the majority of natural color additives. It is concluded that the ingestion of natural color additives presents a very low risk of provoking adverse reactions.

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