

Immune Reactivity to Food Coloring

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ABSTRACT

Artificial food dyes are made from petroleum and have been approved by the US Food and Drug Administration (FDA) for the enhancement of the color of processed foods. They are widely used in the food and pharmaceutical industries to increase the appeal and acceptability of their products. Synthetic food colorants can achieve hues not possible for natural colorants and are cheaper, more easily available, and last longer. However, since the use of artificial food coloring has become widespread, many allergic and other immune reactive disorders have increasingly been reported. During the past 50 y, the amount of synthetic dye used in foods has increased by 500%. Simultaneously, an alarming rise has occurred in behavioral problems in children, such as aggression, attention deficit disorder (ADD), and attention-deficit/hyperactivity disorder (ADHD). The ingestion of food delivers the greatest foreign antigenic load that challenges the immune system. Artificial colors can also be absorbed via the skin through cosmetic and pharmaceutical products. The molecules of synthetic colorants are small,

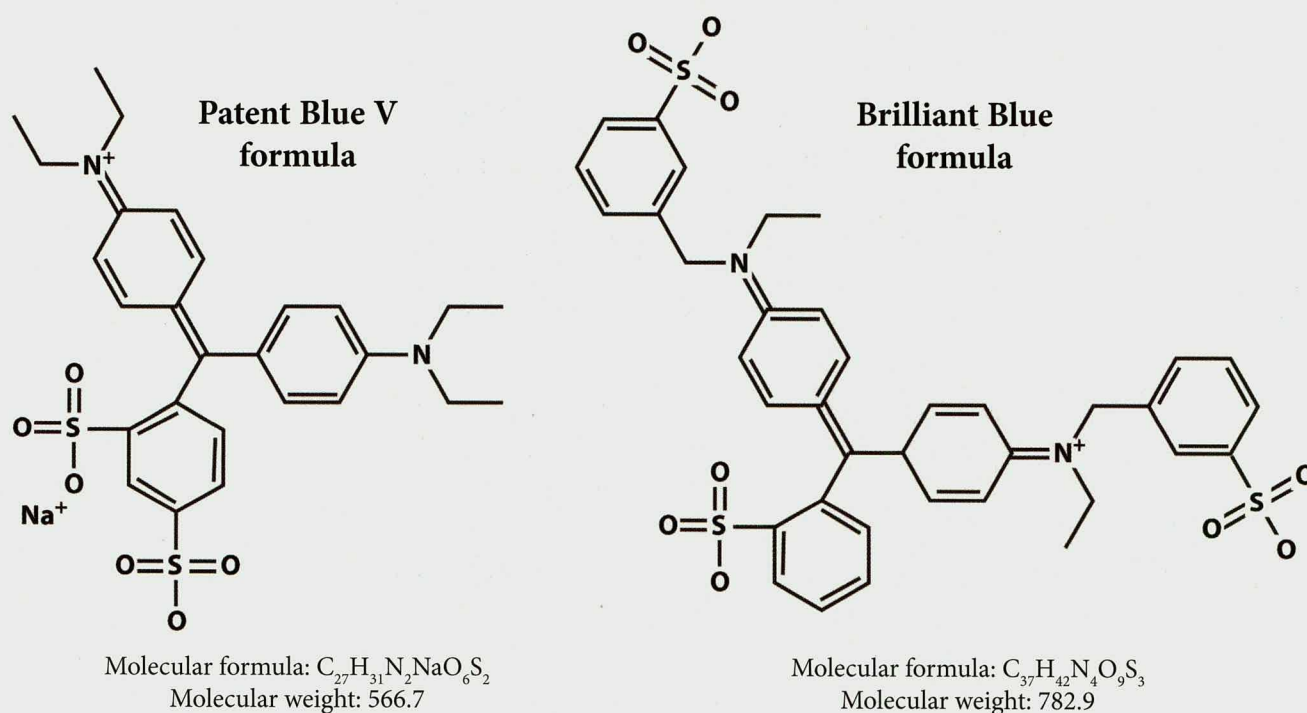
and the immune system finds it difficult to defend the body against them. They can also bond to food or body proteins and, thus, are able to act in stealth mode to circumvent and disrupt the immune system. The consumption of synthetic food colors, and their ability to bind with body proteins, can have significant immunological consequences. This consumption can activate the inflammatory cascade, can result in the induction of intestinal permeability to large antigenic molecules, and could lead to cross-reactivities, autoimmunities, and even neurobehavioral disorders. The Centers for Disease Control (CDC) recently found a 41% increase in diagnoses of ADHD in boys of high-school age during the past decade. More shocking is the legal amount of artificial colorants allowed by the FDA in the foods, drugs, and cosmetics that we consume and use every day. The consuming public is largely unaware of the perilous truth behind the deceptive allure of artificial color. (*Altern Ther Health Med.* 2015;21(suppl 1):52-62.)

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Color is a very important parameter for many foods to increase their appeal and acceptability in the market. For this reason, food dyes are used in the food and pharmaceutical industries to enhance the appearance of colorless products or foods with natural colors that are unattractive.¹ Colorants are also used to attract the attention of specific age groups, particularly children, and to increase product sales. Food colorings have a long history of use throughout the world in raw and processed foods. Both natural and synthetic dyes are used to improve the appearance of products and to increase consumers' demand for them. Compared with natural dyes, synthetic colorants have more extensive applications because of their high stability in light, availability, and lower production costs.^{2,3} Most natural colors belong to either the anthocyanin or the carotene

Figure 1. Molecular formulas for Patent Blue V: $C_{27}H_{31}N_2NaO_6S_2$ (molecular weight: 566.7) and for Brilliant Blue: $C_{37}H_{42}N_4O_9S_3$ (molecular weight: 782.9).



family in general. They are heat-labile but fade during processing. To overcome such losses and, ironically, to give a natural, appealing look to foods, synthetic colors are added.⁴

Patent Blue V and Brilliant Blue

Also called Food Blue 5 or Sulphan Blue, Patent Blue V (PB-V) is a dark bluish, synthetic dye used as a food coloring. The E number for PB-V is E131. This color is used in medicine in lymph node biopsy, in lymphangiography, and in dental staining to show dental plaque on teeth. Together with Brilliant Blue (BB), this substance is used as a dye for textiles, leathers, plastics, and papers and as a colorant in printing inks, paints, and other household products. European regulatory agencies have not established quantitative limits for an acceptable daily intake of PB-V as a cosmetic coloring. As a food-coloring additive, the recommended daily intake is 15 mg/kg of body weight in Europe and many other countries. However, in the United States and a few other countries, PB-V is not authorized for use in food.⁵

Unlike PB-V, BB (E133 or FD&C Blue No. 1) is approved by the US Food and Drug Administration (FDA) for use in food, drugs, and cosmetics products, with an established and acceptable daily intake of 12.5 mg/kg of body weight.⁵ The molecular formulas for both are shown in Figure 1.

Tartrazine

Tartrazine is an artificially synthesized, azo pigment that is also known as FD&C Yellow No. 5 and E102 (Figure 2). Its use is permitted by the Food and Drug Regulations in

Canada, where Health Canada, the country's health body, requires the identification of specific colors on food labels.⁶ The European Food Safety Authority allows for tartrazine to be used in processed cheese, canned or bottled fruit or vegetables, processed fish or fishery products, and wines and wine-based drinks.^{7,8} Tartrazine was banned in Norway and was also banned in Austria, Sweden, and Germany until the ban was overturned by a European Union directive.⁹ In the United States, the FDA requires that the presence of tartrazine be declared in food and drug products and that a warning regarding the colorant's possible allergenic effects on sensitive individuals be displayed.^{10,11} In France, following the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), tartrazine is approved as a colorant in food products, cosmetics, and pharmaceuticals, with a recommended daily intake of 7.5 mg/kg of body weight. It is a color commonly used throughout the world, mainly for yellow, but it can also be used with BB or Green S to produce various shades of green.^{2,12,13} Products containing tartrazine commonly include processed commercial foods that have an artificial yellow or green color or that consumers expect to be brown or creamy looking.

The following is a list of foods that may contain tartrazine:

(1) desserts and sweets—ice cream, ice pops, and popsicles; confectionery products and hard candy, such as gummy bears and marshmallow treats; cotton candy; instant puddings and gelatins; cake mixes; processed pastries; custard powder; marzipan; biscuits; and cookies;

(2) beverages—soft drinks; energy and sports drinks; powdered drink mixes; fruit cordials; and flavored or mixed alcoholic beverages; (3) snacks—flavored corn chips; chewing gum; popcorn, both microwaveable and cinema-popped; and potato chips; (4) condiments and spreads—jam; jelly, including mint jelly; marmalade; mustard and horseradish; pickles and other products containing pickles, such as tartar sauce and dill pickle dip; and processed sauces; (5) other processed foods—cereal, such as corn flakes and muesli; instant or cube soup; rice, such as paella and risotto; noodles; and puréed fruit.

A number of personal care and cosmetics products may contain tartrazine, including (1) liquid and bar soaps; green hand sanitizer; moisturizers and lotions; mouth washes; perfumes; toothpastes; shampoos, conditioners, and other hair products; (2) cosmetics, such as eye shadow, blush, face powders and foundations, and lipstick, even those that are primarily pink or purple (usually the makeup manufacturers use 1 label for all shades in a product line, placing the phrase “may contain” ahead of all colors that are used in that line but not necessarily in the specific shade); and (3) nail polish, nail polish remover, temporary tattoos, and tanning lotions.

Various types of medications also may include tartrazine, primarily for easy identification, to give a yellow, orange, or green hue to a liquid, capsule, pill, lotion, or gel. Types of pharmaceutical products that may contain tartrazine include vitamins; antacids; cold medications, including cough drops and throat lozenges; lotions; and prescription drugs. Most, if not all, data sheets of medications are required to contain a list of all ingredients, including tartrazine. Some include tartrazine in the allergens alert section.²

Other products such as household cleaning products, paper plates, pet foods, crayons, inks for writing instruments, stamp dyes, face paints, and envelope glues may also contain tartrazine.

Allura Red

Allura Red or red azo dye is also known as Food Red 40, FD&C Red No. 40, Food Red 17, E129, or Curry Red (Figure 3).¹⁴ Many products, including medications, hair dyes, and a variety of foods contain Allura Red. The extensive use of Allura Red as a color additive in foods, drugs, and cosmetics indicates that the general population may be exposed to this dye via ingestion of food, beverages, and drugs that use this compound. This use may result in its release into the environment through various waste streams.

In one study, rats were fed a diet containing 5.19% of Allura Red.¹⁴ The researchers observed that 0.1% and 29% of the intact dye was excreted in the urine and feces, respectively. In later studies, rats and dogs were pretreated daily with nonradioactive Allura Red.¹⁴ Subsequently, the animals were dosed with the 35S labeled compound and studied for up to 72 hours for excretion and distribution patterns of the color. Both species showed limited absorption of the compound, with the major route of excretion being via the feces. In the dogs, 92% to 95% of the recovered radioactivity appeared in

Figure 2. Molecular formula for tartrazine: $C_{18}H_{14}N_2Na_2O_8S_2$ (molecular weight: 534.3).

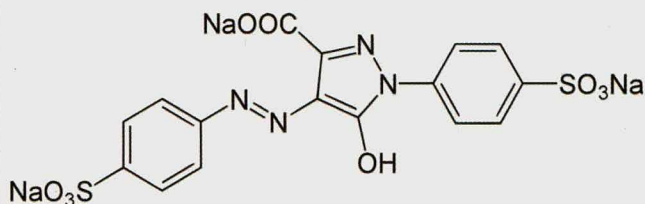
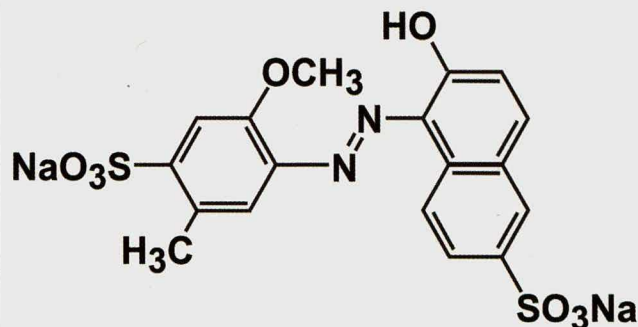


Figure 3. Molecular formula for Allura Red: $C_{18}H_{14}N_2Na_2O_8S_2$ (molecular weight: 496.4).



the feces within 72 hours, whereas in the rats, 76% to 92% of the recovered radioactivity appeared in the feces within that period. Urinary recoveries of the color in rats and dogs varied between 5.7% and 19.8% and between 2.7% and 3.6%, respectively. After sacrifice, significant retention of radioactivity was found in the intestinal contents of both species and in the washed intestines of the rats. This result was thought to be due to adhesion of the compound to the intestinal wall, because the total carcass and viscera of these animals contained <0.4% of the administered dose.

These findings indicate that a significant amount of dyes or their metabolites can bind to human tissue and form neoantigens. Repeated use of foods, cosmetics, or drugs containing colorant can result in bioaccumulation in the tissue; the food coloring plus tissue antigens can then be attacked by the immune system, resulting in autoimmune reactivity.¹⁵⁻¹⁷

Erythrosine

Erythrosine, which is also referred to as FD&C Red No. 3, E127, or Food Red 14, is an iodine-containing, artificial coloring made from coal tar (Figure 4). Erythrosine is also a xanthene dye, which are a group of brilliant fluorescent dyes ranging in color from yellow to pink to bluish red. They are called xanthene dyes because they all contain a xanthene molecule as their base.

The chemical formula for erythrosine is $C_{20}H_8I_4O_5$. Figure 5 shows the structure of the erythrosine molecule. The shared part of the formula (highlighted) shows why erythrosine is categorized as a xanthene dye.^{15,18,19}

Erythrosine is primarily used as a food dye. Some of the more common applications include (1) cocktail and candied

Figure 4. Molecular formula for xanthene: $C_{13}H_{10}O$ (molecular weight: 182.2).

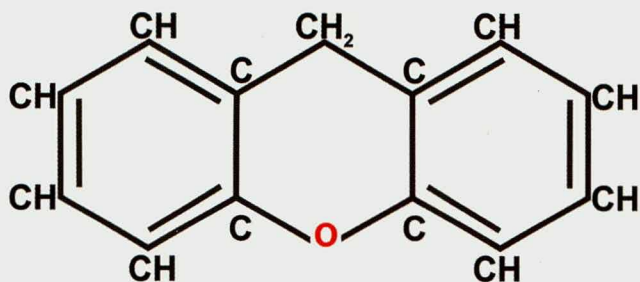
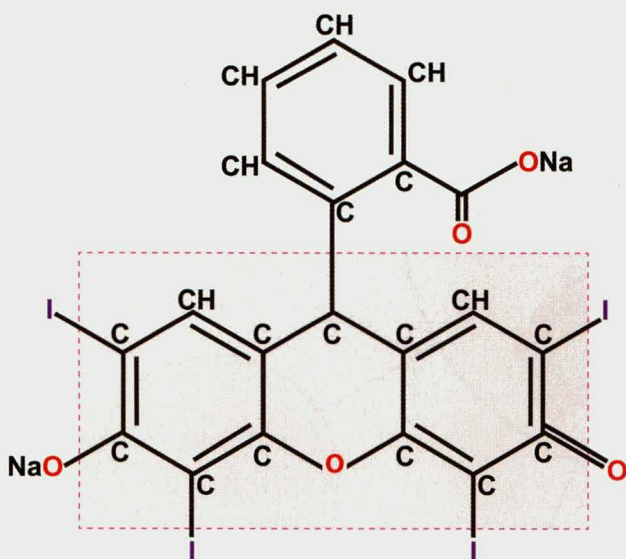


Figure 5. Molecular formula for erythrosine: $C_{20}H_8I_4O_5$ (molecular weight: 879.9).^a



^aThe shared part of the formula (highlighted) shows why erythrosine is categorized as a xanthene dye.

cherries, such as maraschino cherries; (2) candies; (3) popsicles; (4) cake decorating gels; and (5) pistachio shell coloring.

Erythrosine is not used frequently in the United States because Allura Red is used instead. In 2008, the Center for Science in the Public Interest petitioned the FDA for a complete ban on erythrosine, but so far, the agency has taken no action. The colorant can still be used in the United States without restriction. Concerns exist that the iodine may affect the thyroid. Some studies have indicated a higher risk of thyroid tumors in rats.²⁰

HEALTH ISSUES AND USE OF COLORING

Immune and allergic reactions to food additives are a part of food immune reactivities and allergies. Since the use of food additives has become widespread and extensive throughout the world during the past 20 years, many allergic and other

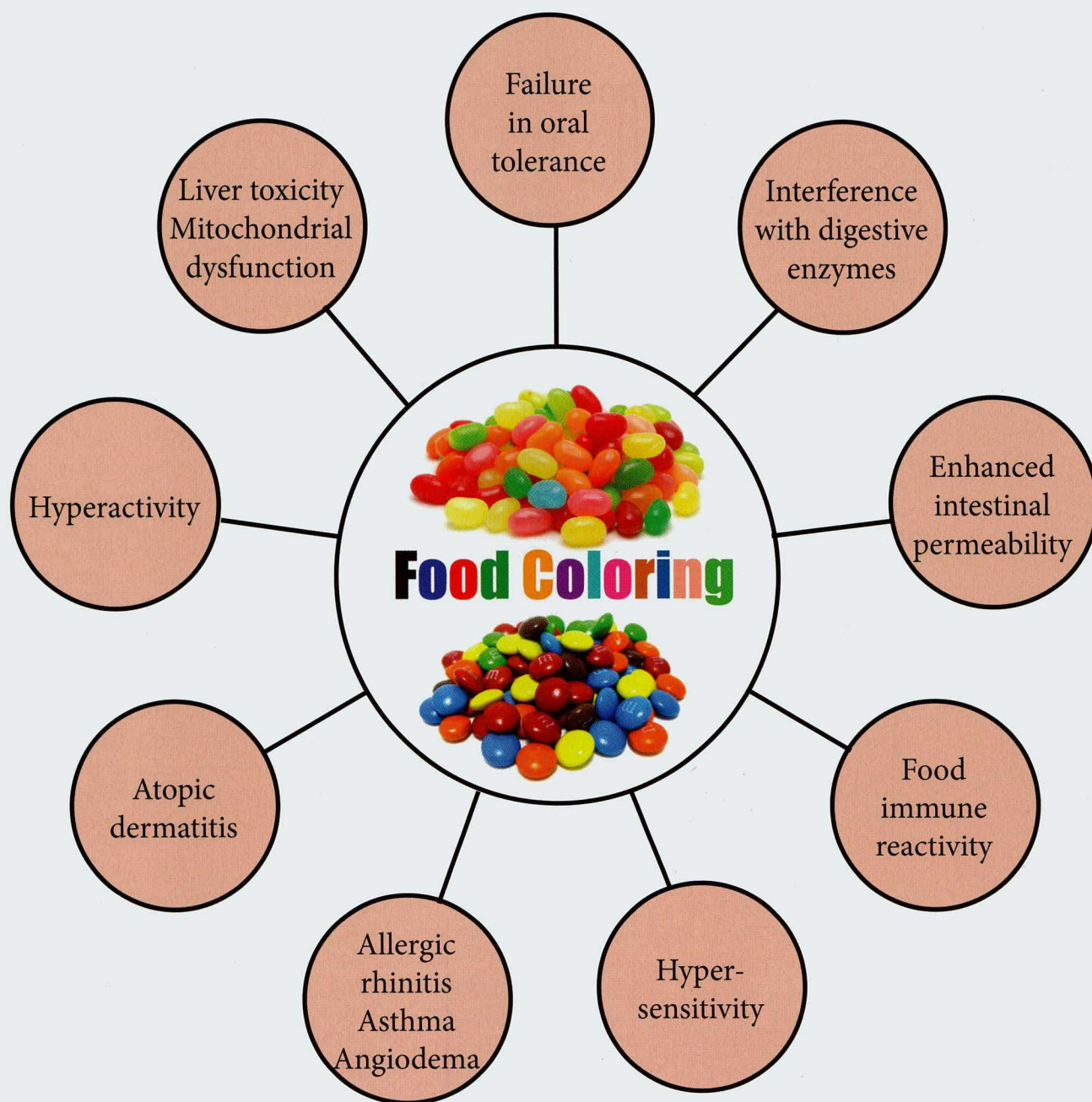
immune reactivities have been reported.² The ingestion of food itself provides the greatest load of foreign antigenic substances that confront the human immune system.

Failure in protective, mucosal immune tolerance against food antigens is the major factor in the induction of immune reactivities and allergies against food antigens. Food coloring and other additives are made of very small molecules, and our bodies do not know how to develop a tolerance against them. If these chemicals manage to bind to food proteins during the manufacturing of foods or to body proteins after metabolization, then these food additives become the major reason for immune tolerance breakdown, and they are able to do so in a stealthy or hidden manner.²¹ For this reason, calculating the prevalence of food immune reactivity and allergy is very difficult. Food allergy or hypersensitivity alone is estimated to be between 3% and 35% of the US population.²²

It is believed that food additives play a significant but hidden role in these immune reactivities.¹⁶ In addition, food coloring, in particular tartrazine, has been suggested as a trigger for asthma and urticarial attacks, particularly in aspirin-intolerant patients.¹³ These reactions have occurred following ingestion of meals or products containing tartrazine and other food colorings. For example, one study investigated 25 patients with clinical symptoms of immunoglobulin E (IgE)-mediated food allergy.¹⁸ This study's participants were challenged orally with various food additives, including tartrazine. In 5 of 25 patients (20%), a positive reaction to tartrazine was obtained by an oral provocation test. However, the study did not assess non-IgE immune reactivities by measuring IgG or immunoglobulin A (IgA) antibodies against tartrazine or other food additives that were bound to human serum albumin (HSA).

An even higher percentage of children with severe atopic dermatitis reacted to tartrazine.²⁴ Tartrazine and other colorants have been reported for their involvement in multiple chemical sensitivities that are not IgE-mediated.²⁵ Many other studies and case reports have associated the use of many food additives other than tartrazine, including Allura Red and erythrosine, to allergic, immunologic abnormalities such as chronic urticaria, angioedema, and rhinitis, which are normally associated with food allergies. For example, when 25 patients with clinical symptoms suggestive of allergy to food antigens were examined by oral provocation test with various food additives, almost 50% of the patients positively responded to 2 additives—sodium benzoate and Sunset Yellow.¹⁸ That study and many others associated with food-coloring immune reactivities were reviewed extensively in a review article,² which concluded that allergic and immune reactivities to food additives can be considered as triggers or aggravating factors in sensitive individuals. Therefore, clinicians and, particularly, sensitive consumers should be aware of the allergic properties of food additives; the removal of these triggers can help to improve a patient's symptomatology immensely.

Figure 6. Disorders associated with food coloring.



BINDING TO HUMAN TISSUE PROTEINS

Whether it is through licking lollipops repeatedly, eating ice cream, using medications, or applying cosmetics, shaving cream, or skin treatments, a significant amount of food additives manage to enter the blood stream through the gastrointestinal (GI) tract.⁵ Many food additives can penetrate into the blood either via skin absorption after application of cosmetics or skin creams or via the GI tract through food intake.^{5,26} Because most food additives carry very active chemical groups, their entry into the human system results in food-coloring interactions with human proteins.

Almost all proteins are made of polar and nonpolar amino acids along polypeptide backbones. Protein molecules also provide a combination of electrostatic and stereochemical interactions. Because food colors are generally ionic in nature, they interact with proteins strongly and form covalent bonds.²⁷ In fact, one of the reasons food additives are used extensively throughout the world is because colors form stable complexes with proteins and give uniform color distribution in all common food systems.^{28,29} This covalent binding of colors to human proteins is a major mechanism for the induction of the immune reactivity and hypersensitivity that is associated with various colorants.³⁰

Figure 7. Formation of tartrazine protein adduct: covalent binding of tartrazine through carboxylic group to amino groups of human serum albumin.

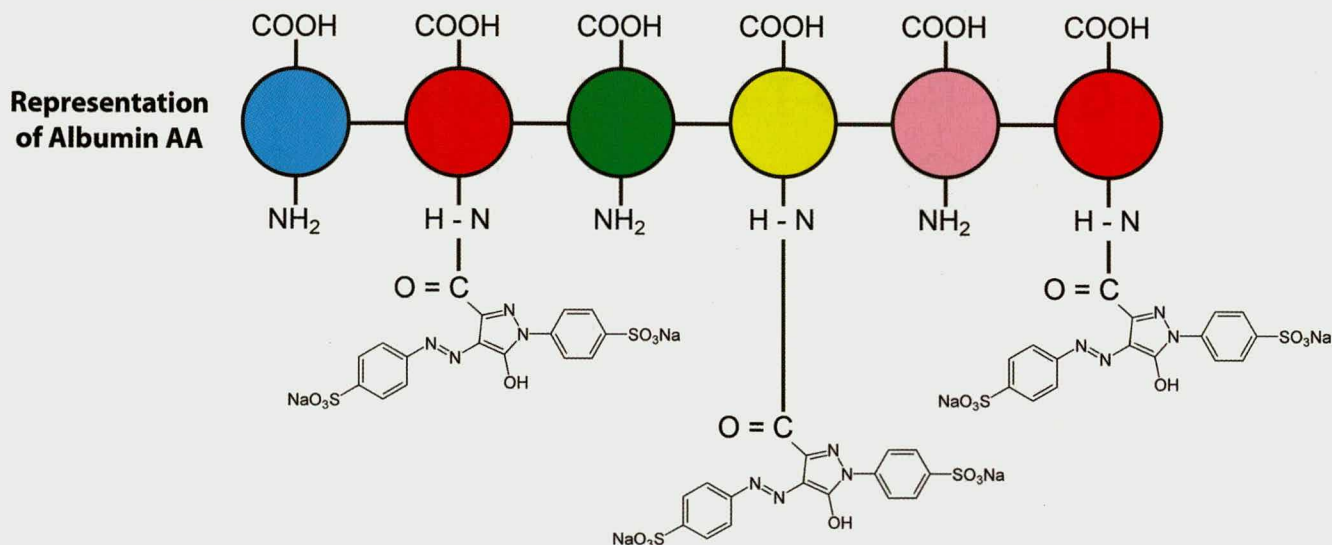


Figure 8. Amino acid 16-40 sequence of albumin; trypsin cleavage is shown by the scissors.



Abbreviations: K, lysine; R, arginine.

In a very recent study,³¹ male and female mice were given 0.45% or 1% of tartrazine through drinking water for a period of 13 weeks, together with a standard food-pellet diet. After 13 weeks of administration and 18 hours of fasting, the animals were sacrificed and histological examinations of the intestine, thymus, and spleen were performed. The histological section of jejunum showed significant lymphocyte infiltration and inflammation in all treated male and female groups. In addition, partial villous atrophy was observed in tartrazine-treated groups. This effect of tartrazine was very pronounced on villus length. For example, in comparison with a villus length of 60 mm for the control group, the length of villi for mice treated with tartrazine was approximately 30 mm, a decrease of 50%.³¹ Based on these results and many other findings that are not discussed here, the researchers concluded that subchronic ingestion of tartrazine at a dose of 1% caused structural alterations of the intestine and spleen, thymus-cell damage, and a depressing effect on the humoral immune response in male mice. It is advisable to limit the usage of synthetic food colorants, particularly for products consumed or applied by children and immunologically vulnerable people.³¹

EFFECTS ON PROTEIN DIGESTIBILITY

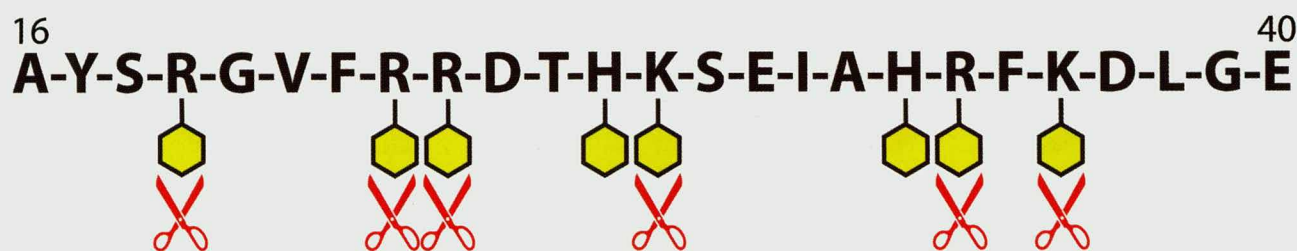
After ingestion, many food proteins are digested by various enzymes and are broken down, first into peptides and then into amino acids, which are absorbed through the gut

barriers. However, a main question in use of food colorings involves the fate of the food-coloring molecules that bind to food proteins during processing versus molecules that are not bound to proteins. Many studies have established that food coloring binds covalently to different body proteins, including HSA and hemoglobin.³²⁻³⁵ These publications show that carmoisine, Allura Red, Sunset Yellow, erythrosine, amaranth, tartrazine, Quinoline Yellow, and BB bind with proteins in a variety of food environments and that the protein color complexes are assumed to be digested by proteolytic enzymes. Because different functional groups of colors bind to the active sites of food proteins, that binding can modify and decrease the tryptic digestibility of the different proteins.³²

This formation of stable complexes between food colors and edible or inedible proteins is shown in Figure 7. Almost all food colors carry very active groups; proteins are made of many amino acids, each containing both amino (NH_2) and carboxylic (COOH) groups. These amino or carboxylic groups are perfect for binding with the colorants' active groups, forming covalent bonds. In Figure 8, the decrease of the digestibility of albumin by trypsin through cleavage of amino acids is shown using an image of a scissors (lysine = K and arginine = R).

Because the active groups of colorants prefer to bind to the lysine (K), arginine (R), and histidine (H) sites of food proteins to form covalent complexes, the digestibility of

Figure 9. Covalent binding of colorants to 3 major amino acids: arginine (R), histidine (H), and lysine (K) to partial sequence of albumin. Note that binding of colors to the protein significantly affects its digestibility.



proteins decreases significantly. This covalent binding of colors to the amino acid sequences of albumin blocks the process of digestion, the cutting capacity of the scissors, as shown in Figure 9.

Indeed, one study showed that 156 of 607 amino acids of bovine serum albumin (BSA) bind with the dyes.³² To show that binding of colors to food proteins interferes with their digestibility, the researchers performed the following experiment. Preparing each solution separately, a solution of BSA proteins was combined with food dye in a concentration of 10 mg/mL of food dye to 0.1 mg/mL of protein. Equal volumes of BSA protein and color solution were mixed in test tubes separately and incubated at 37°C for 2 hours. After combining them, the incubated mixtures of color-bound proteins were digested separately by trypsin, at the enzyme concentration of 1 mg of trypsin to 50 mg of substrate, for various periods of time. After completion of the different periods, the reactions were terminated by adding 1 mL of 10% tricarboxilic acid (TCA) to the respective enzyme, and the undigested proteins were precipitated. The extent of proteolytic activity of the supernatant was measured spectrophotometrically at 280 nm.

As Figure 10 shows, with the addition of the enzyme only to the albumin, the digestion of the protein increased proportionally to the incubation time, but colors binding to the proteins and the formation of color-protein complexes significantly inhibited the ability of the digestive enzyme to digest the albumin.³² It was concluded that the consumption of food colors, particularly synthetic colors, and their binding with body proteins, have significant immunological consequences in the modern lifestyle. If this binding occurs in the digestive tract, the result can be an accumulation of undigested immunogenic peptides in the gut, which can activate the inflammatory cascade. This effect of food colors on tryptic digestibility of dietary proteins, combined with their effects on the villi structure,³¹ can result in the induction of intestinal permeability to large antigenic molecules. If the entry of these molecules through submucosa to the circulation continues, the result could possibly be cross-reactivities and autoimmunities.^{36,37} But if free colors or their metabolites form color-protein complexes or neoantigen formations throughout the body, the result could be autoimmunity against the targeted protein. For example, if colors bind to liver enzymes, the result can be liver

autoimmunity, and if colors form neoantigens with hemoglobin, the result can be the destruction of red blood cells (RBCs), and hence low RBC count.

In a very recent study,³⁵ this toxic interaction of the food-coloring tartrazine with hemoglobin was investigated at the molecular level. The results showed that tartrazine can bind to the active site or the central cavity of hemoglobin to form a tartrazine-hemoglobin complex. The study concluded that tartrazine had an obvious toxic effect on hemoglobin.³⁵ Similarly, the mechanism of the interaction between food dye and HSA was investigated in a physiological buffer³⁸ and the results showed that binding of food coloring to HSA induced conformational changes in the molecular structure of the HSA.

HSA is a major soluble-protein constituent of the circulatory system; it has many physiological and pharmacological functions. For instance, it contributes to colloid osmotic blood pressure and is mainly responsible for the maintenance of blood pH. Further, it can bind and transport a large number of the ligands that are present in blood, such as drugs, bilirubin, bile acids, and metabolites.³⁹ The binding of chemicals to proteins can change macromolecular conformation and, thus, affect the physiological function of proteins. In addition, the binding of coloring to HSA can induce autoimmune reactivity against the protein, which further interferes with its physiological functions. For example, bilirubin, a neurotoxic product of heme catabolism, is detoxified after binding to albumin, which transports it to the liver for further conjugation and excretion in the form of bile.³⁴ Bilirubin-albumin binding is of physiological relevance, and any interference with this interaction may have severe consequences. Unfortunately, several drugs and dyes, including erythrosine, can bind competitively at or near the site of albumin, where bilirubin binds. That binding can displace bilirubin from albumin, resulting in increased blood levels of bilirubin and other serious physiological consequences. In view of these results, the researchers concluded that individuals consuming colored foods and drinks may be at greater risk in developing hyperbilirubinemia.

EFFECTS ON BEHAVIOR

Attention-deficit/hyperactivity disorder (ADHD) affects from 5% to 7% of children worldwide. ADHD is characterized by excessive and impairing inattentive, hyperactive, and

Figure 10. Effect of color binding to bovine serum albumin (BSA) on digestibility by an enzyme. The binding of the colorant to BSA inhibits digestion. The supernatant was measured spectrophotometrically at 280 nM.

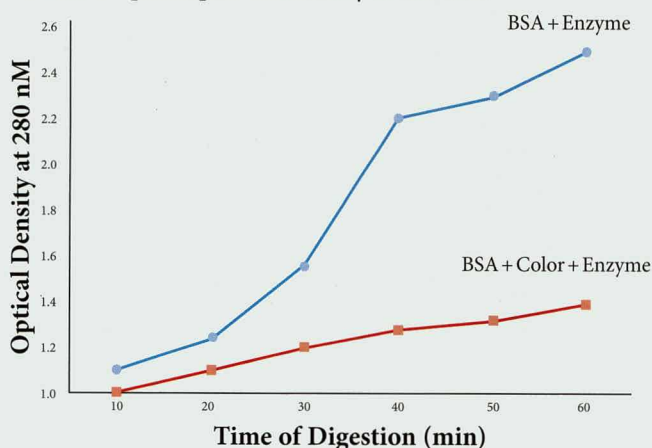
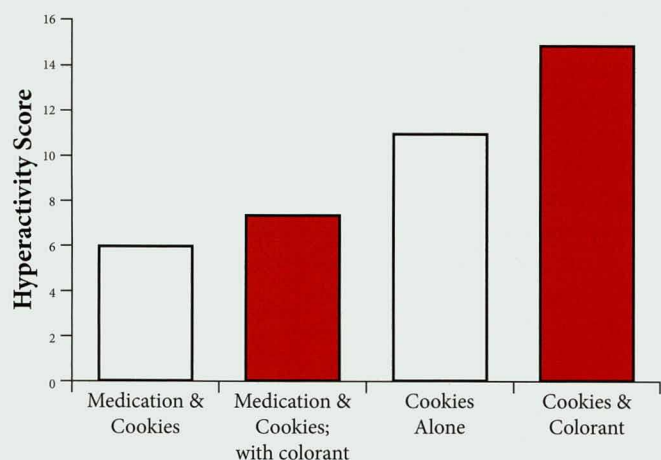


Figure 11. Increase or decrease in hyperactivity scores due to combinations of medication, cookies, and colorant. Modified from Williams et al.³⁵



impulsive behavior.⁴⁰ It is believed that genetics and environmental factors are involved in ADHD and associated disorders.⁴¹ Foods and additives are 2 major environmental triggers that have been studied and that have been found to be associated with such behaviors.⁴²⁻⁴⁴

The effect of food antigens, preservatives, and artificial colors as factors involved in ADHD has been a controversial subject. The effect of foods and additives on learning and behavior was postulated as early as 1922,⁴⁵ but only in 1973 was it hypothesized that foods containing preservatives and salicylates could cause hyperactivity in children.⁴⁶ This theory led to anecdotal reports; only a few appropriately designed, controlled studies have shown any benefits for elimination of artificial colors and foods containing salicylates in the amelioration of hyperactivity in children.^{42,44,47,48}

In a test of Feingold's hypothesis that food additives trigger a hyperactive response, 26 hyperactive children were randomly assigned to treatment conditions in which they

were given active or placebo medications in combination with challenge cookies with artificial colors or control cookies without the additives. The assessment of the children's behaviors was done by the teachers in a blind fashion. The results, summarized in Figure 11, first of all showed that medication and cookies without colorants resulted in a hyperactivity score of 6. When cookies containing colorant were eaten while the participants were using medication, the hyperactivity score went up to 7.4. However, when the drug was replaced with a placebo and cookies with no colorants or cookies with artificial color were given to participants, the hyperactivity score went up to 11 for the cookies without colorants and to 15 for the cookies with artificial colorants. The rating of hyperactivity clearly indicated that the effects of diet were greatest when children did not take medication and that significant reductions in hyperactivity were related to diet in approximately 25% of the children.

Another investigation⁴⁴ evaluated 26 children who met the criteria for ADHD. In an open challenge, 19 (73%) of the children reacted to many foods, dyes, and/or preservatives. Those 19 children responded favorably to treatment with a multiple-item elimination diet ($P < .001$). A double-blind, placebo-controlled food challenge was completed with 16 of the 19 children, where a significant improvement occurred on placebo days when compared with challenge days ($P = .003$). Atopic children with ADHD had a significantly higher response rate to food challenges in the study than the nonatopic group. This study demonstrated a beneficial effect when eliminating reactive foods and artificial colors in the diets of children with ADHD, showing that atopic children—who were known to have sensitivity to food antigens, such as wheat, milk, eggs, and oranges—had a significantly more beneficial response to an elimination diet. Therefore, dietary factors may play a significant role in the etiology of a majority of children with ADHD. The study's findings would seem to indicate that a combination of dietary proteins and additives, or a complex formation between them, plays a significant role in ADHD.

The occurrence of adverse skin, gut, and other physical reactions to foods, such as milk and wheat, has stimulated speculation that such foods could also produce adverse behavioral effects. A double-blind, controlled study has suggested that foods and additives can affect hyperactive behavior adversely.⁴⁹ In this study, 78 children, referred to a diet clinic because of hyperactive behavior, were placed on an elimination diet, removing foods containing additives, chocolate, dairy products, wheat, oranges, tomatoes, and eggs.

The behavior of 59 of the children improved during this open trial. For 19 of these children, it was possible to disguise foods, additives, or both that reliably provoked behavioral problems. The researchers did so by mixing them with other tolerated foods and then tested their effects in a placebo-controlled, double-blind challenge protocol. The results of a crossover trial on these 19 children showed a significant effect for the provoking foods in worsening ratings on behavior and impairing performance on psychological tests.

Reintroduction of 1 or more additive-containing foods resulted in the reoccurrence of symptomatology in 70% of participants. This and earlier studies concluded that clinicians should give weight to the accounts of parents and consider treatment with an elimination diet for children with a suggestive medical history of food immune reactivities, particularly food made with additives.^{43,50}

Similar findings were reported in a recent randomized, controlled trial dealing with the effect of an elimination diet on the behavior of children with ADHD.⁵¹ The impact of nutrition was measured in 100 children with ADHD, of whom 50 were randomly assigned to 5 weeks of a restricted diet (diet group) and the other 50 to a normal diet (control group). Children in the diet group were restricted to a few foods, such as rice, meat, vegetables, pears, and water. All major triggers such as wheat, dairy, food coloring, eggs, and other allergenic food components were eliminated from their diets. After 5 weeks, 78% of those in the diet group had responded to the diet. The assessment of improvement in the children's hyperactivity scores was done both by pediatricians and teachers (Figure 12). The difference in hyperactivity scores between the diet group and the control group was highly significant ($P < .0001$).

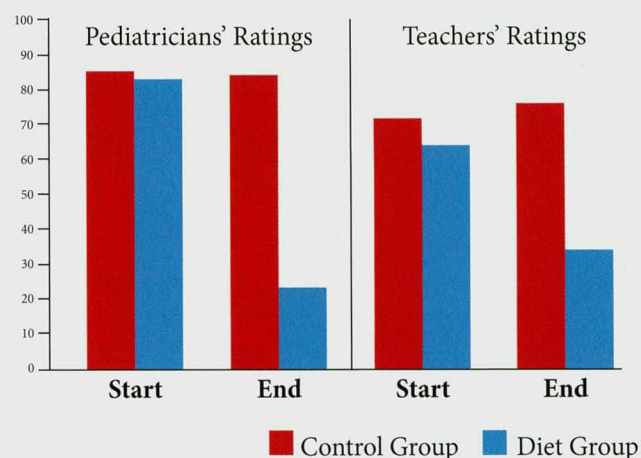
The study showed considerable beneficial effects from a restricted, elimination diet in an unselected group of children with ADHD, with equal effects on ADHD and oppositional defiant disorder. Therefore, the researchers recommended that a dietary intervention should be considered for all children with ADHD, provided that parents are willing to follow a diagnostically restricted elimination diet for a 5-week period and that expert supervision is available. Children who react favorably to this diet should be diagnosed with food-induced ADHD and should enter a challenge procedure to define the foods to which each child reacts and to increase the feasibility and minimize the burden of the diet. In children who do not show a behavioral improvement after following the diet, standard treatments such as drugs, behavioral treatments, or both should be considered.⁵¹

Finally, one study elegantly presented a systemic review and meta-analysis of published works from 1970 to 2013 that had performed randomized, controlled trials of dietary components.⁵² This study has provided much evidence on the harmfulness of food additives for individuals with ADHD. Collectively, exclusion of artificial food colors from the diet of individuals with ADHD had statistically significant effects. As part of clinical guidance about nonpharmacological treatment of ADHD, the researchers concluded that the only dietary or psychological treatments that improved core symptoms of ADHD were supplementation with omega-3/omega-6 free fatty acids and the elimination of artificial food colorings.

SUMMARY AND CONCLUSIONS

Artificial food dyes are made from petroleum and are generally approved by the FDA and similar organizations worldwide for the enhancement of the color of processed foods. They are found in everything from potato chips, soft

Figure 12. Distribution of behavioral scores at the start and end of 5 weeks of a restricted diet, as rated by pediatricians and teachers. Modified from Pelsler et al.⁴⁴



drinks, and puddings to baked goods and even tandoori chicken. During the past 50 years, the amount of synthetic dye used in foods has increased by 500%. Simultaneously, an alarming rise has occurred in children's behavioral problems, such as aggression, ADD, and ADHD.

The Centers for Disease Control recently found that 1 in 5 boys of high-school age and 11% of children overall have been diagnosed with ADHD, which represents a 41% increase in the past decade. In this article, the author has presented data extracted from different scientific articles that show that some association exists between the increased use of petroleum-based food colorings and behavioral problems in children.

After reviewing more than 250 journal articles to write the current review, as an immunologist the author is astounded that these kinds of products remain approved by regulatory officials for human consumption. Further, the author is even more dumbfounded at the amount allowed for human use on a daily basis. For example, officially it is perfectly fine to consume up to 500 mg or 0.5 g of BB or FD&C Yellow No. 5 (tartrazine) on a daily basis. Did you know that if you put 0.5 g of BB or tartrazine in a swimming pool, you can stain the water in the whole swimming pool blue or yellow? Therefore, no one should be surprised by the titles of the following articles, because the author has already shown earlier in the current article that colorants bind covalently to human tissue: (1) "Blue Colon at Autopsy,"⁵³ (2) "Green Colon: An Unusual Appearance at Autopsy,"⁵⁴ (3) "Systemic Absorption of Food Dye in Patients With Sepsis,"⁵⁵ (4) "Toxicity of Food Drug and Cosmetic Blue No. 1 Dye in critically Ill Patients,"⁵⁶ (5) "Reports of Blue Discoloration and Death in Patients Receiving Enteral Feeding Tinted With Dye Blue No. 1,"⁵⁷ (6) "Differential Colon DNA Damage Induced by Azo Food Additives,"⁵⁸ (7) "Reproductive and Neurobehavioral Toxicity of Tartrazine Administered to Mice in the Diet,"⁵⁹ (8) "Reproductive and Neurobehavioral Toxicity of Erythrosine Administered to Mice in the Diet,"⁶⁰ (9) "Effects of Organic Synthetic Food Colours on Mitochondrial Respiration,"⁶¹ (10) "Neurotransmitter Release

from a Vertebrate Neuromuscular Synapse Affected by a Food Dye,”⁶² (11) “Erythrosine B Inhibits Dopamine Transport in Rat Caudate Synaptosomes,”⁶³ and (12) “Immunological Aspects of the Common Food Colorants, Amaranth and Tartrazine.”⁶⁴

Despite all these articles published in scientific journals, the public unfortunately is largely still unaware of the extent of the use of food additives. The author has been equally guilty of being fooled by an attraction to the colors used in various foods. One of his favorite food types is Indian food—particularly the taste, aroma, and color of tandoori chicken. So he enjoyed eating it for many years, thinking that the pink or reddish color on the chicken was from spices such as curcumin, paprika, or cayenne pepper. However, to his surprise, he found that this color is due to the addition of red food coloring. It is a simple fact; if you want your tandoori chicken to be a bright red shade, food coloring is the only way to do it. Only food coloring and not cayenne pepper or paprika can bind covalently to the meat proteins and stain them strongly.

The author is now aware that food coloring can cause the following issues, as shown in Figure 6: (1) a failure in oral tolerance; (2) interference with digestive enzymes; (3) enhanced intestinal permeability; (4) liver toxicity; (5) mitochondrial dysfunction; (6) food immune reactivity; (7) hypersensitivity; (8) atopic dermatitis; (9) allergic rhinitis, asthma, and angioedema; (10) neurobehavioral disorders; (11) interference with neurotransmission; and (12) reproductive abnormalities. Therefore, from now on, he will no longer fall prey to the deceptive lure of the color of his lollipop, medications, sorbet, and soft drinks, or—to his sadness—even his tandoori chicken.

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