

# Anaphylaxis After Ingestion of Carmine Colored Foods: Two Case Reports and a Review of the Literature

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## ABSTRACT

*Two patients with adverse food reactions to foods colored with carmine dye are presented, along with a review of the medical literature addressing adverse reactions to carmine colorant. This review summarizes the mounting evidence suggesting that adverse reactions to carmine colorant are the result of an IgE mediated mechanism. (Allergy and Asthma Proc 20:377-382, 1999)*

Carmine and cochineal extract are biologically derived colorants obtained from the dried bodies of female cochineal (koch-uh-NEEL) insects (*Dactylopius coccus Costa/Coccus cacti L* (Fig. 1). These scale insects belong to the superfamily *Coccoidea*, and reside as parasites on the prickly pear cactus (*Nopalea coccinellifera*). Carmine and cochineal extract impart a pink to magenta red color to the products to which they are added. In the United States, both may be used to color foods and drugs, whereas carmine may also be added to cosmetics.<sup>1,2</sup> Although generally considered safe for human consumption,<sup>3,4</sup> there is now a growing awareness of hypersensitivity reactions related to the ingestion

of carmine colorant, a substance with a rich and fascinating history.<sup>5</sup>

A naturally derived pigment, carmine has been in use for several thousand years. Europe was first introduced to carmine by Hernando Cortés in 1519 after he discovered its use by the Aztecs. Proving to be a valuable commodity for Spain, the dye from the New World found wide ranging applications, including the coloring of textiles, artists pigments, cosmetics, and foods.<sup>5</sup> Still in use, carmine colorant has been linked to many adverse reactions including cheilitis,<sup>6</sup> occupational asthma,<sup>7-11</sup> gastrointestinal complaints,<sup>8</sup> extrinsic allergic alveolitis,<sup>12,13</sup> urticaria/angioedema,<sup>14-21</sup> and anaphylaxis.<sup>18-21</sup>

A small but growing number of case reports and studies have appeared in the medical literature addressing carmine related adverse reactions. Although the incidence of carmine hypersensitivity is unknown, it seems to be rare. And although it is known that carminic acid (Fig. 2) is the coloring principle of carmine,<sup>22</sup> the antigenic component of this colorant remains undefined. Evidence supporting an IgE mediated mechanism as the cause for identified anaphylactic episodes associated with the ingestion of carmine colored foods has come from studies that have demonstrated positive skin prick tests (SPTs),<sup>7-11,15-21</sup> a positive Prausnitz-Kustner (P-K) test,<sup>17</sup> a positive basophil histamine release assay,<sup>15</sup> IgE RAST<sup>7,16,18</sup> studies, and SDS PAGE with IgE immunoblot.<sup>11</sup>

The following describes two patients with episodes of urticaria, angioedema, and anaphylaxis after the ingestion of carmine containing products, as well as a review of the medical literature addressing adverse reactions to carmine

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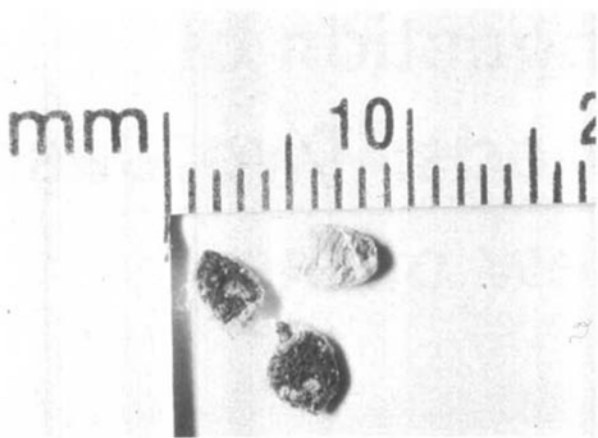


**Figure 1.** Dried bodies of female cochineal insects (*Dactylopius coccus* Costa/*Coccus cacti* L) Scale in millimeters. Fully developed females approximately 6 mm (L)  $\times$  4.5 mm (W)  $\times$  4 mm (H) before dehydration, resulting in estimated 70% loss in mass.<sup>22</sup>

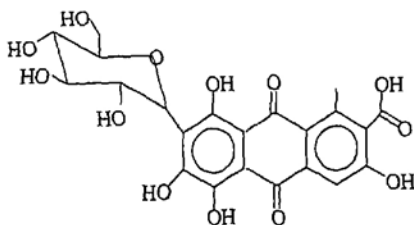


**Figure 2.** Carminic Acid:  $C_{22}H_{20}O_{13}$  (MW 492.39). The coloring principle of carmine, carminic acid is a hydroxyanthraquinone linked to a glucose unit. Carminic acid comprises approximately 10% of cochineal and 2–4% of its extract.

and work done to elucidate the component(s) of carmine responsible for these events.

## CASE REPORTS

### Patient 1

A 27-year-old, nonatopic woman developed anaphylaxis after the ingestion of Yoplait custard style strawberry and banana flavored yogurt, which listed carmine on its list of ingredients. This patient had no other significant past medical history but presented with a history of six discrete episodes of generalized urticaria associated with perioral and periorbital angioedema as well as nausea, diarrhea, and abdominal cramps within the past 3 years. Each episode occurred consistently between 30 minutes and 2 hours after p.o. intake of a substance colored with carmine. Her most severe episode was treated in an emergency department, where a tryptase level was drawn. During her treatment, she was found to be tachycardic (HR = 125 bpm) and hypotensive (BP = 86/palp. mm/Hg). The patient was treated with subcutaneous epinephrine, IV fluids, diphenhydramine, and steroids. She was discharged to home in stable condition 8 hours after presentation. Of interest, this patient has also experienced intense periorbital pruritis and swell-

ing within several minutes of applying carmine containing eye shadow.

### Patient 2

A 42-year-old nonatopic, nonasthmatic female nurse with no known drug allergies presented with a history of multiple episodes of facial angioedema and nasal congestion occurring 30 minutes to 2 hours after food ingestion. The patient recalls developing her first episode at age 28 after the ingestion of imitation crab meat. This patient has required three emergency department visits for severe reactions to foods over the past 10 years. The first of the three emergency department visits was prompted by the onset of nausea, vomiting, diarrhea, itchy palms, facial angioedema, and wheezing 30 minutes after the ingestion of Campari liqueur, which is colored with carmine. The patient was treated with subcutaneous epinephrine, IV fluids, diphenhydramine, and steroids. She recalls being tachycardic, with a heart rate greater than 120 bpm. An association between carmine ingestion and the remaining two emergency department visits could not be confirmed. This patient had also suspected food allergy to walnuts, peanuts, strawberries, and tomatoes. Additionally, she has experienced upper and lower eyelid swelling, facial urticaria, and intense rhinorrhea within 5 minutes of applying eye shadows listing carmine as an ingredient.

## MATERIALS AND METHODS

### Allergens

Full strength carmine liquid color (CLC) and annatto were obtained courtesy of Warner Jenkinson Company, Inc., (St. Louis, MO). CLC diluted in sterile saline was used for all carmine skin prick testing. Additionally, carmine liquid color was centrifuged at 7,500 g for 2 hours in a Centricon 3,000 molecular weight cutoff centrifugation cartridge to obtain a filtrate (molecular weight 3000 or less).

Many commercially available inhalant and food allergen extracts obtained from Greer Laboratories, Inc. (Lenoir, NC), as well as suspected foods were also tested.

### Skin Prick Testing

Skin Prick Testing (SPT) was performed in the customary fashion using the Duotip-Test (Lincoln Diagnostics, Inc., Decatur, IL). All testing was performed on the patients' backs after appropriate preparation by alcohol swab. Cutaneous skin test reactions were graded in comparison to both negative and positive controls. Saline was used as a negative control, whereas histamine chloride 1 mg/mL was used as a positive control.

### Other

Double-blind, placebo-controlled oral challenge (DBP-COC) was declined by both patients.

The patient described in Case 1 had a tryptase level drawn during her emergency department visit within several

hours of the onset of symptoms described above. The tryptase level was sent to the Mayo laboratory (Rochester, MN).

## RESULTS

### Patient 1

SPT to inhalant allergens including tree, grass and weed pollen, as well as dust mites, molds and animal danders were negative (Table I). The patient was skin prick tested to a number of items including full strength carmine color, three 10-fold dilutions of carmine, carmine filtrate, and food items including gum (Nabisco Fruit Stripe), and yogurt (Yoplait Strawberry & Banana, Custard Style and Lite). Some of the items were known to contain carmine, whereas others were known to be carmine free. These items were selected because they were either known or suspected by the patient to be offending agents. SPT was positive to Yoplait yogurt colored with carmine and Fruit Stripe gum containing carmine, but not to Yoplait yogurt without carmine. SPT to carmine in four 10-fold dilutions, as well as carmine filtrate, were also positive. The patient responded appropriately to positive and negative controls, histamine, and saline, respectively. A tryptase level drawn approximately 3 hours after the onset of the most recent anaphylactic episode, occurring after carmine ingestion, was found to be elevated at 18 mcg/L [5.6–13.5 mcg/L]. With strict avoidance of carmine-containing foods and cosmetics, the patient has experienced no adverse reactions in the past 22 months.

### Patient 2

SPT to inhalants including molds, cockroach, and dust mites were negative (Table II). Positive and negative controls responded appropriately. SPTs to peanut, walnut, tomato, and annatto were negative, whereas SPTs to carmine diluted 1:100 and carmine filtrate demonstrated 4+ positive reactivities. Within the past 18 months, several accidental carmine ingestions have caused mild reactions characterized by nasal congestion, and pruritis of the palms

TABLE II

Summary of SPT Results for Patient 2

| Substance                    | Prick | Wheal (mm) | Erythema (mm) |
|------------------------------|-------|------------|---------------|
| Carmine (1:100)              | 4+    | 11         | 62            |
| Carmine filtrate (≤3,000 MW) | 4+    | 10         | 30            |
| Annatto                      | 0     | –          | –             |
| Peanut                       | 0     | –          | –             |
| Walnut                       | 0     | –          | –             |
| Tomato                       | 0     | –          | –             |
| Saline                       | 0     | –          | –             |
| Histamine                    | 3+    | 7          | 30            |

and scalp. Self-treatment with 75 to 100 mg of oral diphenhydramine was reported to be of significant benefit.

### Controls

Forty-two control subjects were SPT negative to carmine at concentrations ranging from 1:10,000 to full strength. Of the 42, 29 (69%) were SPT positive to at least one inhalant allergen and considered atopic on this basis. Ten control subjects were SPT negative to carmine filtrate.

## DISCUSSION

### Carmine/Cochineal Usage

Cochineal extract is the concentrated solution remaining after alcohol is removed from an aqueous-alcohol extract of cochineal insects. Carmine is a water dispersible powder consisting of an aluminum or calcium-aluminum lake on an aluminum hydroxide substrate of carminic acid. Carmine dye is used in several forms depending on the coloring, solubility, and stability characteristics desired (Table III). It may be used as an insoluble lake to provide the reddish hue on the outer surface of imitation crab meat (surimi seafood) without diffusion of the color throughout

TABLE I

Summary of SPT Results for Patient 1

| Substance                           | Prick | Wheal (mm) | Erythema (mm) |
|-------------------------------------|-------|------------|---------------|
| Carmine (full strength)             | 4+    | 20         | 50            |
| Carmine 1:10                        | 4+    | 15         | 35            |
| Carmine 1:100                       | 4+    | 11         | 32            |
| Carmine 1:1000                      | 4+    | 7          | 28            |
| Carmine filtrate (≤3,000 MW)        | 4+    | 20         | 45            |
| Yogurt with carmine                 | 3+    | 7          | 18            |
| Yogurt without carmine              | 0     | –          | –             |
| Fruit Stripe Gum (contains carmine) | 2+    | 5          | 11            |
| Saline                              | 0     | –          | –             |
| Histamine                           | 3+    | 7          | 30            |

TABLE III

| Carmine/Cochineal Usage                                                                                                                                          |                                                                                                                                                                                                                                                                                 |                                                                                                                                                                           |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Water Insoluble Carmine Colors                                                                                                                                   | Water Soluble Carmine Colors                                                                                                                                                                                                                                                    | Water Soluble Cochineal                                                                                                                                                   |
| <ul style="list-style-type: none"> <li>• Cosmetics</li> <li>• Pharmaceuticals</li> <li>• Dairy products</li> <li>• Baked goods</li> <li>• Confections</li> </ul> | <ul style="list-style-type: none"> <li>• Yogurt</li> <li>• Ice cream</li> <li>• Fruit based drinks</li> <li>• Beverages</li> <li>• Fruit fillings</li> <li>• Puddings</li> <li>• Bakery mixes</li> <li>• Confections</li> <li>• Cosmetics</li> <li>• Pharmaceuticals</li> </ul> | <ul style="list-style-type: none"> <li>• Beverages</li> <li>• Yogurt</li> <li>• Ice cream</li> <li>• Fruit fillings</li> <li>• Puddings</li> <li>• Confections</li> </ul> |

the seafood product. It may also be rendered soluble for use in beverages such as Campari liquor, or fruit based drinks, dairy products such as yogurt, ice cream, or puddings. Or it may be added to bakery mixes or baked goods, confections, cosmetics, and pharmaceuticals.

### Labeling and Regulation

According to current labeling guidelines established by the United States Food and Drug Administration (FDA), carmine containing products are required to carry one of the following descriptors in the list of ingredients, "carmine," "cochineal extract," "color added," "artificial color," or "artificial color added" (21 CFR sec 101).<sup>2</sup> Outside the United States, foods containing carmine may be labeled E120 or natural red 4. Although no daily FDA consumption limit has been set, the World Health Organization has set a daily consumption limit at 5 mg/kg/day.

### EVIDENCE FOR AN IgE MEDIATED MECHANISM

#### Basophil Histamine Release Assay

Beaudouin et al. described the first case of carmine-colored yogurt induced anaphylaxis in 1995.<sup>15</sup> The authors estimated the quantity of carmine dye their patient ingested before anaphylaxis to be 1.3 mg. In addition to reporting a positive SPT to carmine powder and carmine colored yogurt, they also reported a positive leukocyte histamine release test. The authors acknowledge that although this provides evidence for an IgE mediated mechanism, only IgE immunoblot would discern between a low molecular weight component acting as a hapten versus a protein acting as the offending antigen.

#### Prausnitz-Kustner (P-K) Test

In 1997 Baldwin et al. reported a patient with carmine dye-induced anaphylaxis after ingestion of a popsicle.<sup>17</sup> The patient's SPTs were strongly positive to the popsicle and carmine. A successful P-K test was performed, transferring carmine specific IgE to the patients, noncarmine-

allergic husband, converting his SPTs to both the popsicle and carmine. Appropriate negative controls were performed, providing strong evidence for an IgE mediated mechanism.

### IgE RAST

In 1997 Wüthrich, Kägi, and Stucker reported five women, ranging in age from 25 to 43, all of whom experienced anaphylaxis after the ingestion of Campari Bitter, an orange colored liquor.<sup>18</sup> Each of the five patients was reported to be SPT positive to carmine. Four of the five patients were atopic by history and SPT to common inhalants. Each of the five women was eventually found to have a positive RAST test to carmine. Three of the five were also RAST tested to carminic acid bound to human serum albumin (HSA), a hapten-albumin conjugate. Of the three, only one was found to test positive. Kägi et al. first described one of the five cases above three years earlier.<sup>16,19</sup> Serologic testing of the first patient was initially negative, but revealed specific IgE to carmine (class 2 positive) by RAST after a 1-year period of repeated carmine exposures.

Quirce et al. in 1994, used RAST and RAST inhibition studies to evaluate the sera of 10 nonatopic individuals who worked in a factory that produced mainly carmine.<sup>7</sup> Other natural dyes, including annatto and turmeric, were also handled. One of the individuals was a former employee who had left after being diagnosed with occupational asthma. DBPCOC was negative in that individual. Three of the 10 subjects, including the former employee, demonstrated positive SPTs to carmine. Only the former employee demonstrated significant drops in peak expiratory flow rates (PEFR) when working with carmine. RAST testing demonstrated specific IgE antibodies against carmine in only the former employee. None of the subjects demonstrated reactivity by RAST testing to carminic acid-HSA. RAST inhibition results indicated that the main allergen was in the 10 kD to 30 kD range.

## SDS-PAGE and IgE Immunoblot

Acero et al. have described a case of occupational asthma and food allergy due to carmine.<sup>10,11</sup> In their study, a 37-year-old nonatopic male working in a spice warehouse demonstrated positive findings to SPT, bronchial provocation test (BPT), and DBPCOC test. The patient's serum was studied using SDS-PAGE with immunoblotting in an attempt to identify carmine-specific IgE antibodies to specific carmine related proteins. Protein bands at 50 kD and 28 kD were identified by SDS-PAGE when an extract of minced cochineal insects was electrophoresed. The authors did not report finding protein bands using carmine colorant. Immunoblotting with patient serum resulted in binding to a great number of high molecular weight bands, suggesting a problem with nonspecific binding. No data on controls were presented.

We have described here two patients with type I hypersensitivity reactions after carmine ingestion. An elevated serum tryptase level was documented during the anaphylactic episode associated with carmine ingestion in one of the patients.<sup>21</sup> Both demonstrated positive SPTs to carmine and carmine filtrate ( $\leq 3,000$  mw), raising the possibility that a small molecular component of carmine may be acting as a hapten.

## CONCLUSIONS

The case studies presented here add to the growing number of case reports in the medical literature of urticaria, angioedema, and anaphylaxis attributable to an IgE mediated allergic reaction caused by the ingestion of carmine. Evidence supporting an IgE mediated mechanism as the cause for anaphylactic episodes associated with the ingestion of carmine colored foods has come from studies that have demonstrated positive SPTs, an elevated tryptase level, a positive P-K test, a positive basophil histamine release assay, IgE RAST studies, and SDS PAGE with IgE immunoblot.

The antigenic determinant(s) of carmine remain unknown. Although it is likely that a residual insect derived protein acting as an allergen is the cause of these hypersensitivity reactions some data, including our own, suggest that a hapten bound to a high molecular weight carrier may also be playing a role in some patients.

Because carmine colorant is capable of producing profound hypersensitivity reactions in concentrations found in ordinary foods and cosmetics, the possibility of carmine hypersensitivity should be entertained in patients who suffer from episodic urticaria, angioedema, or anaphylaxis associated with the ingestion or use of artificially colored products. However, because carmine is classified in the United States by the FDA as "exempt from certification" consumers may have difficulty identifying items colored with carmine. Exempt colors are regulated by the Code of Federal Regulations (CFR) Title 21 Part 73. Section 101.22 of the

CFR states that carmine containing food items may be labeled "colored with carmine," "carmine color," "cochineal extract," "color added," "artificial color," or "artificial color added." Although cosmetics containing carmine are required to specify this ingredient by name, this labeling requirement is usually adhered to with the confusing designation "may contain . . ." followed by an exhaustive list of colorants. This "all inclusive" labeling tactic is equally distressing to consumers attempting to identify carmine free cosmetics.

Labeling changes that would require carmine, a known antigen, to be specifically labeled on lists of ingredients would be of significant diagnostic benefit to physicians who evaluate allergy patients. Equally important, such labeling changes would allow carmine allergic consumers to practice improved avoidance, critical because accidental ingestion of this usually benign substance can lead to anaphylaxis.

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