

Case report

Anaphylactic reactions to ingested carmine (E120)

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We report five cases of anaphylactic reaction to carmine (cochineal, E120) after patients drank an alcoholic beverage. By means of positive skin prick tests (SPT) and positive RAST to carmine, IgE-mediated sensitization could be established. One nonatopic patient showed also a great amount of serum IgE antibodies to the carmine acid-albumin conjugate. Due to its widespread use in the food and cosmetic industry, carmine should be tested in the allergy work-up in case of allergic reactions after a drink or a meal.

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Carmine is a naturally derived red dye extracted from cochineal (E120, color index no. 75470). Cochineal is the dried, pulverized bodies of the female mealybug (*Dactylopius coccus* or *Coccus cacti*) that lives on the cochineal cacti (*Opuntia coccinellifera*) widespread in Mexico, Honduras, Peru, the Canary Islands, Algeria, Spain, and India (1). The brilliant red color of carmine is due to the carminic acid secreted into intracellular vesicles of female *C. cacti*. Carmine is mainly used for coloring beverages (e.g., Campari Bitter®), food, medicine, and cosmetics (lipstick, eye shadow) and as a biologic dye. In 1994, we described a severe anaphylactic reaction in a woman to Campari-Orange, which proved to be IgE-mediated (positive skin prick test [SPT] and RAST) to carmine (2). Since this first report of food anaphylaxis after ingestion of carmine, another case of food allergy after eating yogurt containing mixed fruits and the dye carmine has been described (3). This case also provided evidence of an IgE-dependent mechanism (positive prick test and leukocyte histamine-release test with carmine). The amount of carmine contained in the yogurt was estimated to be 1.3 mg. Recently, a severe and prolonged asthmatic reaction after heavy occupational exposure to the food color

cochineal/carmine was described in a flavorer working in a food factory (4). An isolated IgE-mediated reaction to carmine was proved by skin tests and RAST determinations. Furthermore, using RAST, the authors were able to detect IgE antibodies to carminic acid, the color component in carmine. In the past 2 years, we diagnosed four new cases of carmine allergy to Campari, and performed *in vitro* allergy tests by RAST, not only with the whole carmine preparation but also with carminic acid (bound to human serum albumin [HSA]), in order to determine whether also in the case of ingested carmine, carminic acid must be considered the specific allergen.

Material and methods

Skin tests

SPT were performed with routine inhalant and food allergens (Alyostal Extracts, Stallergènes, France), a standardized needle (Stallerpoint, France) being used. SPT were also performed with a drop of undiluted Campari Bitter, the carmine dye (kindly provided by the manufacturer, Campari SA, Milan, Italy) being diluted 1 : 1 with saline, and

with a commercially available carmine 0.5% solution (Brial Allergen GmbH, Greven, Germany). Histamine 1 mg/ml was used as positive and glycerol as negative control. The results of the SPT were evaluated by the EAACI guidelines (5). Ten atopic controls were negative to Campari and both carmine preparations with the SPT.

Serum IgE

Total and specific serum IgE, including Phadiatop or SX1-Mix as *in vitro* screening test for atopy, was determined by the ImmunoCap tests (IgE-FEIA and RAST-FEIA, respectively; Pharmacia CAP-System, Uppsala, Sweden), according to the manufacturer's recommendation.

Allergen disks

Cyanogen bromide-activated paper disks (6, 7) were used as solid phase for 1) carmine, the Campari coloring material (Campari, Milan, Italy) (2); 2) cochineal/carmine (23.2% water-soluble powder) (Xantoflor, Tuleda, Spain) (4); and 3) carminic acid (Sigma C 3522), bound to HSA.

Since carminic acid contains a carboxylic group (Fig. 1) available for coupling, EDC-mediated immunogen formation is the method of choice for hapten-carrier conjugation (8). Therefore, this molecule was conjugated to the carrier-protein HSA, as described elsewhere (4). For typical conjugation, the hapten carminic acid and the protein HSA (Sigma A8763) are incubated with EDC (1-ethyl-3-(3-dimethylaminopropyl)carbo-diimide-hydrochloride) (Sigma E7750) in MES-buffer (2-[*N*-morpholino]-ethanesulfonic acid) (Sigma M5287) at pH 4.5 for several hours.

The hapten-protein complex is purified and buffer-exchanged by column gel filtration using Sephadex G25 gel (Pharmacia Fine 17-0032-01) and carbonate-bicarbonate buffer at pH 8.6.

The increase of the absorbance at 280 nm was used to control the conjugation. A control experiment without EDC was also performed for the reaction.

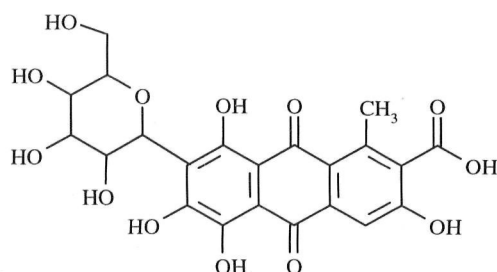


Fig. 1. Structural formula of carminic acid.

RAST performance

For establishment of the diagnosis of IgE-mediated allergy to carmine, the patient sera were first tested by RAST with disks kindly prepared with carmine (Campari SA, Milan, Italy) by Prof. S. G. O. Johansson, Department of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden, as described elsewhere (2). The results were expressed in RAST class and Phadebas RAST units/ml (PRU/ml), respectively (6).

RAST with these new disks was performed by incubating them with 50 µl of serum for 3 h at room temperature. The disks were then washed three times with PBS buffer, each time with 2 ml of the washing solution. After the washing procedure, 50 µl of ¹²⁵I-labeled anti-IgE (Dr Fooke Laboratories GmbH, Neuss, Germany) with an activity of about 50000 cpm was added, and the disks were incubated overnight at room temperature. After 20 h, unbound, radiolabeled anti-IgE was removed by washing as described before, and the samples were counted for 1 min in a gamma counter (Hybritech Europe SA, Liège, Belgium).

The results were expressed as percentage of the total activity added to each sample. All assays were done in duplicate. The final results were calculated in units/ml (u/ml) by means of a reference standard curve and were assigned to classes 0 to 4 according to the RAST scale.

Case reports

Case 1

At a garden party, a 34-year-old atopic woman, with a history of allergic rhinoconjunctivitis and mild allergic bronchial asthma, had a severe anaphylactic reaction 15 min after drinking Campari-Orange. The details of the history and results of allergy tests have been previously reported (2). The RAST to carmine was initially negative (despite clearly positive SPT with both Campari and carmine), but became positive (class 2, 0.8 PRU/ml) on the occasion of a follow-up after a year during which the patient repeatedly suffered from minor allergic episodes, probably due to undeclared red-dyed food, such as ice cream.

Case 2

A 33-year-old woman who had suffered from rhinoconjunctivitis symptoms from the early spring until late summer since the age of 8 years was referred for allergologic investigation because she had experienced several episodes of acute urticaria and angioedema, usually after a meal,

and twice after drinking Campari-Orange. She also reported oral allergy syndrome to apple, peach, and nuts. The allergy SPT showed polyvalent sensitization to pollen of hazel, alder, birch, ash, grass, and mugwort. SPT with commercial extracts was negative except for celeriac and camomile (Allergopharma GmbH, Reinbeck, Germany), but prick-prick tests with fresh food were strongly positive for apple, hazelnut, carrot, celery, and potato. The SPT with Campari dye was ++ (wheal 6 mm), and with carmine 0.5% (Brial) + (wheal 3 mm). The total IgE was 570 kU/l, CAP-positive class 2 (0.93 kU/l) for apple, but negative for other food. The RAST to carmine (Prof. S. G. O. Johansson) was class 1 (0.67 PRU/ml). The patient was instructed about her sensitization and given an emergency kit. In the following year, she experienced once an allergic reaction with swelling of the underlip and generalized itching, 30 min after a Mexican rice meal with shrimp and several sauces. New allergy tests with food were negative, but the carmine RAST was now class 2 (0.8 PRU/ml) positive.

Case 3

A 43-year-old patient without obvious atopic diseases was seen by a dermatologist (Dr P. Schmid, Rüti) because she had suffered a very severe anaphylactic reaction which required inpatient treatment in the hospital. In fact, 15 min after drinking nonalcoholic beer and three sips of Campari Bitter, she experienced itching eyes and rhinorrhea, followed by enormous swelling of the eyelids, generalized itching with urticaria, and dyspnea. The hospital doctors felt that the non-alcoholic beer was the cause of the allergic reaction, because soon after drinking that beer, the patient had developed sneezing. However, 1 year later, after drinking one glass of Campari-Orange – without food – she again experienced 15 min later eyelid edema, urticaria, and dyspnea. The SPT were positive only for mugwort (++), camomile (++), and celeriac (+) (Allergopharma). A prick and a scratch test with Campari were strongly positive, but negative with beer. After the testing, the patient suffered nausea and red and watery eyes. The tests in the allergy unit in Zurich confirmed the sensitization to mugwort, camomile, and carmine from Campari, but not that from the Brial manufacturer. The total IgE was 540 kU/l, and the CAP for mugwort was class 2 (1.2 kU/l) positive. The carmine RAST (Prof. S. G. O. Johansson) was class 2 (2.4 PRU/ml), clearly positive. RAST with various spices, including celery salt, aniseed, fennel, and caraway, were all negative.

Case 4

A 25-year-old woman from the USA, with a history of allergic rhinoconjunctivitis caused by cats and dogs, developed, 30 min after a meal of lamb, curry, rice, and Campari-Orange, sneezing, rhinitis, nasal obstruction, redness in the face, angioedema, widespread urticaria, and dyspnea. She had emergency treatment with epinephrine by Medihaler and endovenous antihistamines in the department of dermatology in Zurich. The allergy tests performed a few weeks later demonstrated only sensitization in the SPT to cat and dog epithelia. SPT with food, including lamb, rice, and spices, were all negative. SPT were positive with Campari (+) and with Campari dye (++), but negative with carmine (0.5% Brial).

The total IgE was 68 kU/l, the specific IgE (CAP) was class 2 for cat dander (0.74 kU/l) and class 1 for dog epithelium (0.46 kU/l), and the tests were negative for mutton and bovine serum albumin. RAST for carmine (Prof. S. G. O. Johansson) was class 2 (1.0 PRU/ml) positive.

Case 5

A 39-year-old nonatopic woman developed, 30 min after drinking Campari-Orange and dancing, acute urticaria with angioedema of the face. SPT with inhalants and several foods were negative, SPT with carmine from Campari was negative at 15 min, but ++ positive (wheal 5 mm) at 30 min, and a scratch test was ++ at 15 min, but the SPT with carmine 0.5% (Brial) remained negative. The total IgE was 85 kU/l, and the inhalant (SX1) and the food (fx5) mix were negative in CAP. The carmine RAST was class 1 (0.68 PRU/ml).

Results

The patient data and the results of the initial allergy tests are listed on Tables 1 and 2, which show the results of the RAST determinations with the different carmine and carminic acid preparations in sera from cases 1, 4, and 5, as well as in the serum of the patient with occupational asthma due to inhaled carmine (4). Only the serum in case 5 presented IgE antibodies not only to carmine, but also to carminic acid.

Discussion

All our five patients with a history of allergic reaction after drinking Campari demonstrated by SPT and RAST sensitization to the red dye carmine (E120). They were all women, three with a history of allergic respiratory disease and one with

Table 1. Carmine allergy: patient data

Case no.	Age (years)	Sex	Atopic diseases	IgE (kU/l)	Phadiatop/ Sx1 (CAP)	Carmine RAST*		SPT with carmine	
						PRU/ml	(class)	From Campari (1:1 H ₂ O)	From Brial (0.5%)
1	34	F	RCPo, ABPo		Positive	0; 0.8	(0); (2)	++	+
2	33	F	RCPo, OAS	570	Positive	0.67; 0.8	(1); (2)	++	+
3	43	F	No**	540	Positive	2.4	(2)	++	0
4	25	F	RC (cat, dog)	68	Positive	1.0	(2)	++	0
5	39	F	No	85	Negative	0.68	(1)	++	0

RCPo=rhinoconjunctivitis to pollen; ABPo=asthma bronchiale to pollen; OAS=oral allergy syndrome; RC=rhinoconjunctivitis. * Prof. S. G. O. Johansson, ref. 2. ** SPT positive to mugwort, but without clinical symptoms.

Table 2. RAST results with different carmine/cochineal preparations and with carminic acid in three patients and three controls

	Carmine from Campari		Carmine/cochineal		Carminic acid-HSA	
	U/ml	(class)	U/ml	(class)	U/ml	(class)
Case no. 1	<0.35	0	0.56	1.7	<0.35	0
Case no. 4	1.56	2.4	1.53	2.3	<0.35	0
Case no. 5	0.56	1.7	0.80	2.0	0.49	1.5
Control 1 (serum 1994)	0.8	2.1	1.08	2.3	1.10	2.3
Control 2 (serum 1996)	6.74	3.1	7.34	3.1	11.86	3.4
Negative control	<0.35	0	<0.35	0	<0.35	0

only nonclinical sensitivity to mugwort, but one was obviously nonatopic. Due to the clear relationship between ingestion of Campari and skin test positivity to Campari and carmine, they all refused oral challenge, but they agreed to blood examination. None of them had occupational or inhalative exposure to carmine powder. In our first case, the sensitization was probably due to the use of eye shadow, since, when the patient had used this product before the severe anaphylactic shock after drinking Campari, itching in the eyes and burning skin developed (2). SPT with this cosmetic were also positive (2).

In fact, the first description of an allergic reaction to carmine, in 1961, was as contact allergy (allergic cheilitis) to lip salve containing carmine, with positive patch tests in three affected patients (9). Interestingly, the first case of anaphylactic shock to carmine resulted from casualty simulation in a soldier while a makeup stick containing carmine red was being applied to his trunk (10). Occupational asthma secondary to inhalation of carmine powder was first described in 1979 (11), and later in 1987 (12) and in 1994 with demonstration of specific IgE antibodies against carmine and cochineal (13). A case of extrinsic allergic alveolitis secondary to occupational carmine exposure with positive serum precipitins against carmine was published 1991 (14). Finally,

Stücker et al. detected specific IgE antibodies to carminic acid in a patient with severe asthma reaction after heavy exposure to the food color cochineal (4). Therefore, carmine may act as potent contact, inhalant, and food allergen and elicit all three types of allergic reactions (IgE-, IgG-, and T-cell-mediated). As already stated (3), carmine should not be confused with cochineal red A (E124, color index no. 16255) (synonymy: new coccin, ponceau 4R, or food red 7), a synthetic azo-dye. With the latter, pseudoallergic urticarial reactions have been detected in children by oral challenge tests (15).

All our five patients had positive SPT and specific serum IgE to the carmine/cochineal extract, kindly supplied by Campari, Italy. Cochineal extract is the concentrated solution obtained after removing the ethanol from an aqueous ethanolic or ethanolic extract of cochineal, which consists of the dried bodies of the female insect *D. coccus* Costa. It requires about 70 000 insects to make 500 g of cochineal. The coloring principle is chiefly carminic acid (Fig. 1). Commercial products also contain proteinaceous material derived from the source insect. Three sera could also be examined for IgE antibodies to carminic acid-HSA. The two atopic sera were negative; the nonatopic one (case 5), however, was positive. Control sera of the nonatopic patient with occupational inhala-

tive exposure to carmine/cochineal showed a great amount of IgE antibodies to this hapten-albumin conjugate. One can speculate whether in atopic patients the ingestive exposure to carmine/cochineal leads to an IgE response mainly to cochineal proteins, eventually due to cross-reactivities to other inhalant sources. However, in our four atopic patients, there was not a clear cluster of reactivities; two of them were polyvalently sensitized to pollens, one had isolated sensitization to mugwort, the fourth was sensitized to cat and dog epithelia, and none were positive to the red *Chironomus* larva (*Chironomus thummi thummi*, i 73) (data not shown), so that the reaction seemed to be quite specific. Due to widespread use of carmine in the food and cosmetics industry, one can speculate that unclear episodes of anaphylactic reactions (so-called idiopathic anaphylaxis) (16) may be due to such a sensitization. Therefore, carmine should be tested in the allergy work-up of a "restaurant syndrome" (17) or in the case of allergic reactions after a meal.

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