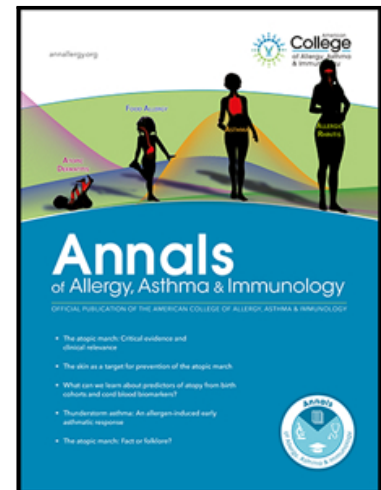


Annato hypersensitivity after oral ingestion confirmed by placebo-controlled, oral challenge.

Beata Sadowska , Marlena Sztormowska , Marta Che Imińska

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Title: Annatto hypersensitivity after oral ingestion confirmed by placebo-controlled, oral challenge.

Authors: Beata Sadowska, MD; Marlena Sztormowska, MD; Marta Chełmińska, PhD

Department of Allergology, Allergology and Pneumonology Clinic, Medical University of Gdansk,
Smoluchowskiego 17, 80- 214 Gdansk, Poland

Corresponding author: Beata Sadowska, MD, Department of Allergology, Allergology and Pneumonology Clinic, Medical University of Gdansk, Smoluchowskiego 17, 80- 214 Gdansk, Poland;
tel. +48 58 584 43 00, fax: +48 58 584 43 10, E-mail: sadowska.beata@yahoo.pl

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Natural dyes, in contrast to synthetic ones, can be a source of protein, so their consumption may be associated with IgE-dependent allergic reactions. Among natural dyes, symptoms were most often observed after carmine ingestion¹ and only occasionally after annatto and concerned the skin, respiratory system or even anaphylaxis. Annatto (E-160b) is a natural yellow-orange dye obtained from the seeds of the tropical tree *Bixa orellana*, coming from Americas.² It is found as a colorant in many foods, such as cheese, margarine, breakfast cereals, grilled chicken, dressings, coffee creamers, mustard, colored rice, popcorn, ice cream, crackers, yoghurts, cakes, jelly and medicines. The acceptable daily intake of E-160b is 2.5 mg / kg body weight / day. Annatto is also used in the production of cosmetics, such as creams, shampoos, body lotions, that are considered natural or vegan, in which it can be described as the color index (CI) 75120.

Only a few cases of annatto hypersensitivity have been published so far. Nish et al. described the patient with urticaria and anaphylaxis after consuming breakfast cereals confirmed by a positive skin test results.³ Ebo et al. presented a case of 56-year-old atopy patient with recurrent anaphylaxis after consuming Gouda cheese containing annatto. The source of anaphylaxis was confirmed based on the history, positive skin prick tests with annatto and cheese and basophil activation test.⁴ Stein described a patient, who developed abdominal pain, bloating and diarrhea after consuming coffee cream and vanilla ice cream containing annatto.⁵ Myles et al. linked recurrent urticaria in a 2-year-old patient with consumption of annatto colored cheese.⁶

Here we report the first case of annatto hypersensitivity in the form of contact urticaria and oral ingestion. The patient was a participant in a study conducted at the Allergology and Pneumonology Clinic, Medical University of Gdańsk, Poland concerning the evaluation of hypersensitivity to food additives among patients with urticaria. The study protocol was approved by the Bioethics Committee (NKBBN/546/2016-2017) and met the ethical standards of Helsinki Declaration.

A 34-year-old female patient was hospitalized due to recurrent urticaria for three years. She was treated for asthma, hypothyroidism and underwent tonsillectomy, cesarean section and *Helicobacter pylori* eradication. In addition, the patient reported skin eczema after contact with metal. The urticaria wheals were usually generalized, except for the hands and genital areas, occurred up to four times a year, lasted for three days, and usually resolved with a triple daily dose of fexofenadine. The patient did not report any other accompanying symptoms apart from severe itching. She mentioned exposure to a spray tanning, self-tanning lotion, sweets and fabric softener as the culprit factors. Stress and heat were additional co-factors aggravating the symptoms. Laboratory tests revealed a negative *Helicobacter pylori* antigen, TSH value at the lower limit of normal and no eosinophilia. The skin prick tests with aeroallergens showed sensitivity to house dust mites, while the test with autologous serum was negative.

Skin prick tests performed with 1.2% annatto, 1% carmine and 1% sodium metabisulfite were negative. Patch test results, with 1% carmine and 1% annatto on petrolatum, read at 48, 72, 96 and 168 hours were negative. The rationale behind conducting these tests as part of the project was to check for sensitivity to food additives, that were consumed by the patient and could potentially cause allergic reactions. Based on an analysis of composition of tan lotion and spray tanning agent used in the beauty studio, the presence of annatto in both products was found.

Following written consent, the patient was exposed to single blinded placebo controlled oral challenge with increasing annatto doses ranging from 1 to 100 mg according to the challenge protocol provided. Capsules with 1/2/5 mg were administered every 20 minutes, and subsequent doses of annatto 10/20/50/100 mg were given every hour (Fig. 1). Annatto was delivered by Bart LTD, Słupno, Poland. After the administration of the 20 mg dose a slight cleavage erythema with mild transient itching was reported. After one hour, when the symptoms had completely disappeared, the challenge was continued. One hour

after 100 mg dose administration, the patient developed massive urticaria. A gradual reduction of symptoms was achieved after 20 mg of cetirizine and 60 mg of prednisone administration. Rigorous avoidance of cosmetics and food with annatto resulted complete resolution of symptoms during next year. At the next visit, both skin prick test and patch test with 10% powdered annatto extract concentration were performed and they appeared negative. Then we decided to conduct a second challenge, which resulted in generalized urticaria after a annatto dose of 50 mg.

This is the first published case that illustrates the occurrence of contact urticaria after annatto ingestion. Although it was not possible to establish allergy to E-160b in skin tests, which were positive in other reports,^{3,4} a suggestive history with a positive two oral challenge tests points to annatto as the culprit of contact urticaria and oral hypersensitivity. Therefore non-IgE mediated mechanism was possible in this case. The patient did not take antihistamines on a regular basis, therefore the occurrence of urticaria could not be associated with the abrupt discontinuation of antiallergic treatment.

During our research project, none of the 110 studied patients with urticaria showed positive annatto skin prick and patch tests. Bearing in mind that we do not know the concentration of annatto in cosmetics and a significant contact skin exposure to allergen during spray tanning, it cannot be ruled out that reaction was dose-dependent.

The small number of annatto hypersensitivity reports may be due to insufficient awareness of the possible allergy to natural dyes. The growing tendency to replace synthetic dyes with natural dyes may result in a higher percentage of allergies in the future, as their sources are plant (e.g. annatto) or animal (carmine) allergens. If hypersensitivity to annatto is suspected, oral challenge should be considered despite negative skin testing.

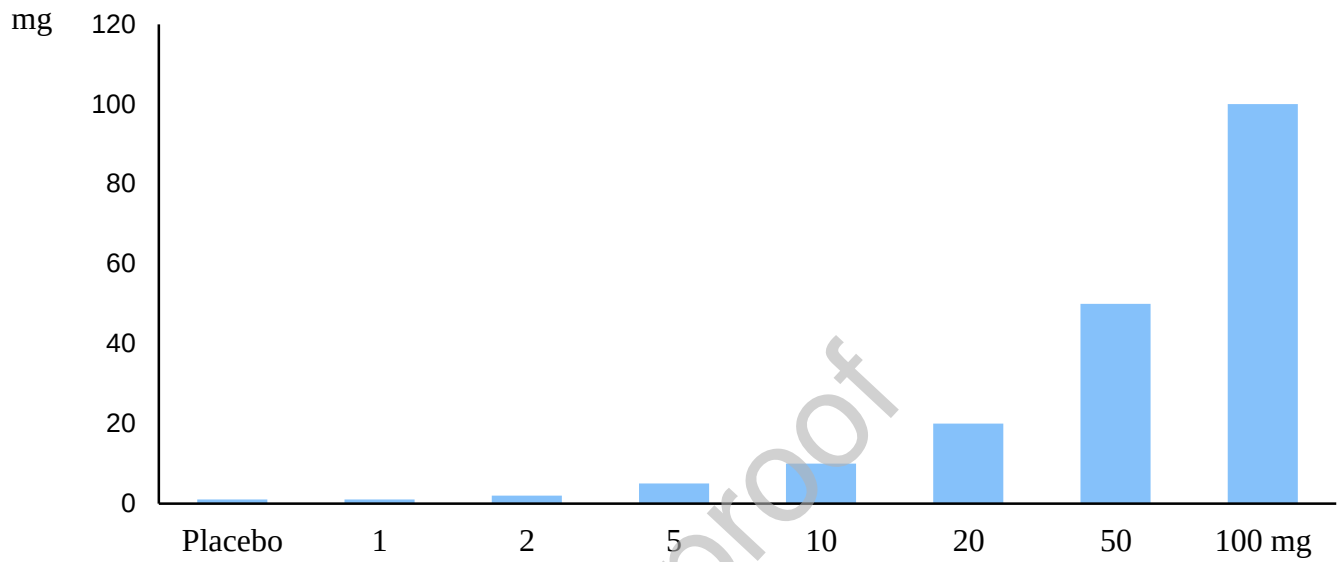
In summary annatto may act as an allergen or a hapten, mediating IgE-mediated allergic reactions, or alternatively may act as an IgE-independent mast cell activation

mechanism. However, the pathophysiological mechanisms underlying these reactions remain unknown.

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Fig. 1. Annato single blind placebo controlled oral challenge protocol.



dosing intervals:

-----every 20 min-----//-----every 1 hour-----