

Hydrolysed wheat proteins present in cosmetics can induce immediate hypersensitivities

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Cosmetics containing hydrolysed wheat proteins (HWP) can induce rare but severe allergic reactions. 9 patients, all females without common wheat allergy, but with contact urticaria to such cosmetics, were studied. 6 of them also experienced generalized urticaria or anaphylaxis to foods containing HWP. All patients had low to moderate levels of immunoglobulin (Ig)E specific of wheat flour (f4) or gluten (f79). Their sensitivity to HWP and their tolerance to unmodified wheat proteins extracted from grains were confirmed using skin tests. Immunoblotting analyses showed that IgE from all patients reacted with almost all HWP tested. Reactions generally occurred with large random peptide aggregates. IgE reacted also with unmodified grain proteins, which contrasted with skin tests results. They reacted always with salt soluble proteins but variably with gluten proteins. No reaction occurred with gliadins in patients without associated immediate hypersensitivity to food containing HWP. These results show the role of hydrolysis on the allergenicity of wheat proteins, both through skin or digestive routes. At least part of the epitopes involved is pre-existing in unmodified wheat proteins. The aggregation of peptide bearing these epitopes and others created by hydrolysis, along with the increased solubility and the route of exposure, are possible factors of the allergenicity of HWP.

Key words: allergenicity; contact urticaria; cosmetics; hydrolysed wheat proteins; protein aggregates. © Blackwell Munksgaard, 2006.

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Wheat protein derivatives are widely used in the composition of current products of western lifestyle. Several isolated cases of contact urticaria due to hydrolysed wheat proteins (HWP) in cosmetics (1–4) or of anaphylaxis to deamidated gluten in food (5) have already been described. This type of pathology differs from other forms of immediate hypersensitivity to wheat proteins, now well documented and reviewed by Palosuo (6). HWP are prepared either from insolubilized total flour proteins or more generally only from gluten. Wheat flour proteins are a complex mixture of soluble proteins of albumin and globulin type and of insoluble structural and storage proteins. These last proteins are divided into gliadins, which are monomeric, and glutenins, which are highly polymeric. Gluten results from the association of these two protein groups, along with some carbohydrates and lipids. Industry uses gluten either with or without modifications. The main modification is hydrolysis, to overcome its insolubility. Hydrolysis is performed either in

acid conditions or more and more by using enzymes. The procedure used and the degree of hydrolysis depend on the desired functionality and on the manufacturer.

We describe here a set of 9 patients with contact urticaria to cosmetics containing HWP. A rapid description of the history of the first patient has already been presented (3). It was also mentioned along with 6 additional patients in a poster summary (4). Some of the patients also reacted to foods containing these HWP. The 9 patients were all tolerant to traditional wheat products, bread, pastries, etc. Their detailed skin reactivity with different HWP and wheat flour proteins is now compared to the specificity of their serum immunoglobulin (Ig)E, for these products.

Materials and Methods

Material and reagents

Reagents were, unless specified, of analytical grade and from Sigma (Sigma-Aldrich Corp.,

St. Louis, MO, USA) or Pierce (Pierce Biotechnology, Rockford, IL, USA). Wheat grains and flours were additive free. HWP and normal gluten were obtained either from the food or cosmetic manufacturers or directly from the producer. No details could be obtained on the hydrolysis procedures. A set of 3 different HWP, identified here by A, B and C, was used to characterize the patients. They came from different producers. A second set of 2 acidic and 1 enzymatic HWP of the same gluten source, from producer D, was also used.

Patients

9 women (21–53 years old), having used HWP-containing products, were identified by the co-authors, among patients with either suspicion of food allergy or presenting only dermatologic lesions. 6 patients were identified at Tenon hospital (Paris), 2 at Saint Eloi hospital (Montpellier) and 1 at Saint-Jacques hospital (Besançon), in the period 2000–2004. All patients were tested according to the same protocol. They were evaluated clinically and by skin tests (open tests and if negative, prick tests) with (a) the suspected cosmetics, (b) the corresponding HWP it contained, supplied by the manufacturer, (c) the suspected food and the corresponding HWP, in case of associated food allergy to such products, and (d) with wheat flour extract (Stallergenes S.A., Antony, France). Additionally, for some patients, prick tests were performed with well-characterized wheat protein fractions (discussed below). For these last tests, protein fractions issued from 10 mg of flour were dispersed in 1 ml of glycerol-saline solution and then diluted in the same medium. Increasing concentrations, from dilution 10^{-3} to pure mother solution, were applied on the skin, depending on the patient's reactivity. These last tests received the consent of the patients and the approval of the local Ethical Committee. Specific IgE to wheat flour (f4) and gluten (f79) was also determined (UniCap[®] Pharmacia Diagnostics AB, Uppsala, Sweden).

Wheat protein preparations

Wheat protein fractions were extracted from pure flour without additives, according to their solubility characteristics: albumins and globulins were soluble in salt solutions, while gluten proteins were precipitated by salt. Among them, gliadins were soluble in ethanol 65% (w/w), and low and high molecular weight glutenin subunits (LMW-GS and HMW-GS) were solubilized successively in 65% (w/w) 1-propanol, 1% (v/v) 2-

mercaptoethanol and 35% (w/w) 1-propanol, 0.1 M acetic acid and 5% (v/v) 2-mercaptoethanol. Acid and alkaline hydrolyses were performed on aliquots of these fractions by heating them in 0.1 M HCl or NaOH, for 1 hr in a boiling water bath. After neutralization, they were lyophilized. Controls without proteins were performed in the same time.

Analytical methods

All sera were analysed in the same conditions. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on Novex NuPAGE[®] 4–12% gels, using Z-(morpholino) ethane sulfonic acid (MES) buffer, according to the manufacturer specifications (Invitrogen Corp., Carlsbad, CA, USA). Separated proteins were blotted in semi-dry conditions (7) on polyvinylidene fluoride (PVDF) membranes (Millipore Corp., Billerica, MA, USA). They were stained on polyacrylamide gels using Coomassie blue (8) and on PVDF membranes using Indan ink (9). For IgE immunodetection, membranes were incubated overnight with patient sera diluted 10 times. IgE fixation was revealed using secondary rabbit anti-human IgE conjugated to horseradish peroxidase (Daco SA., Trappes, France) and the West Dura extended duration kit (Pierce Biotechnology, Rockford, IL, USA). Chemiluminescence was recorded on Kodak X-Omat AR5 films, after few minutes of exposure.

Results

Patients' histories

The 9 patients were identified among patients with either suspicion of food allergy or presenting only dermatologic lesions. None of them were primarily consulting for contact urticaria. They, however, had in common to have used cosmetics containing HWP and to have experienced reaction with them. They did not react with any of the allergens commonly known to be present in cosmetics. They also experienced from 1 to more than 10 attacks of contact urticaria immediately after applying a cosmetic containing HWP (Table 1). For some patients, the cosmetics had been applying for a few months before the onset of contact urticaria. The identified cosmetics were mainly skin moisturizers: facial creams ($n = 15$), less often body moisturizing cream ($n = 3$), shampoo and hair conditioner ($n = 2$) and shower gels ($n = 1$). They came from 11 different brands. For 2 patients, the cosmetics were not identified (samples in beauty shops). 3 of the 9 patients were atopic (allergic rhinitis,

Table 1. Description of the patients included in the study

Patient number	Age (years)	Atopy	Number of attacks	Cosmetics type	Reactivity to food with HWP	Food type	Positive skin test	Specific IgE	
								Wheat flour (f4) (kUA/l)	Gluten (f79) (kUA/l)
1	48	Astma	>10	Facial cream, body moisturizer	Generalized urticaria	Preserved food, ham	Cosmetics, foods, HWP	16.4	16.2
2	21	Rhinitis	>10	Facial cream ×>3, body moisturizer	Anaphylaxis	Liver pâté, Pâté foie gras.	Cosmetics, foods, HWP	<0.35	<0.35
3	25	0	3	Facial cream	Anaphylaxis	Pâté	Cosmetics, foods, HWP	<0.35	<0.35
4	51	0	>3	Facial cream ×2, body moisturizer	0	–	Cosmetics, HWP	<0.35	<0.35
5	35	0	?	Facial cream, shower gel	Generalized urticaria	Ham	Cosmetics, foods, HWP	1.46	0.89
6	36	0	>3	?	Generalized urticaria, WDEIA	Preserved food, Pâté foie gras, Canellonis	Cosmetics, foods, HWP	0.61	2.16
7	28	0	3	Facial cream, shampoo, hair conditioner	Angiodoema	Liver pâté HWP	Cosmetics, foods,	<0.35	ND
8	53	0	3	Facial cream ×2, hair conditioner	0	–	Cosmetics, HWP	ND	ND
9	39	Rhinitis	>5	Facial cream ×4	0	–	Cosmetics, HWP	<0.35	<0.35

HWP, HWP contained in the cited products; WDEIA, wheat dependent exercise induced anaphylaxis; ND, not determined for this patient.

$n = 2$ and asthma, $n = 1$). 6 of them experienced also generalized urticaria ($n = 3$), anaphylaxis ($n = 2$) or angiodoema ($n = 1$) when eating preserved food or delicatessen which contained HWP. For these patients, reactions to cosmetics preceded food reactions. No patient had allergic reactions after eating bread, traditionally baked products or pasta.

Patient testing

All patients developed positive skin prick tests with the suspected cosmetics and the HWP used in the cosmetic and with the suspected food and the identified HWP used in it, in case of food allergy (Table 1). Skin tests were negative with wheat flour extract (Stallergenes S.A., Antony, France). Levels of wheat flour (f4) and gluten (f79) specific IgE were significant only in patients 1, 5 and 6 (Table 1). Five patients had skin tests under a controlled protocol, with purified wheat protein fractions, unmodified, acid and alkaline hydrolysed (Table 2). Again no skin reaction occurred with the unmodified proteins. Positive skin reactions developed with most hydrolysed

protein fractions. The pH of hydrolysis had almost no effect, excepted for patient 9, who did not react with alkaline hydrolysed proteins. Similarly, HWP from producer D were tested on patients 4, 6 and 7 (results not shown). These 3 patients developed skin reactions with the two acidic HWP. Only patient 6 developed reactions with the enzymatic HWP.

Characterization of hydrolysed gluten and wheat protein fractions

The original SDS-PAGE pattern of grain proteins was undetectable in HWP samples (Fig. 1, lanes 1–3). Instead, Coomassie blue staining showed only smears, characteristic of random degradations and rearrangement of peptides. Indian ink, which is more sensitive and produces negative staining with some proteins, displayed within the smears some unidentified bands. Smears spanned most of the separation range of the gel, from 3.5 kDa to several 10th kDa. On the contrary, grain proteins showed characteristic bands in the range, 6–116 kDa (lanes 5–9).

Table 2. Skin reactivity of 5 patients with unmodified and hydrolysed wheat protein fractions

Patients	Isolated wheat protein fractions								Wheat extract (Stallergenes)
	Unmodified				Acid/alkaline hydrolysed				
	Alb-Glob	Gliadins	LMW-GS	HMW-GS	Alb-Glob	Gliadins	LMW-GS	HMW-GS	
4	0	0	0	0	+/+	+ /0	+/+	0/0	0
6*	0	0	0	0	+/+	+/+	+/+	+/+	0
7*	0	0	0	0	+/+	+/+	+/+	+/+	0
8	0	0	0	0	+/+	+/+	+/+	+/+	0
9	0	0	0	0	+ /0	+ /0	0/0	0/0	0

Symbols 0 and + mean, respectively, negative, positive skin prick tests; +/+, 0/0 or +/0 compare reactions to acid and alkaline hydrolysed proteins.

*Patients with associated food allergy to hydrolysed proteins.

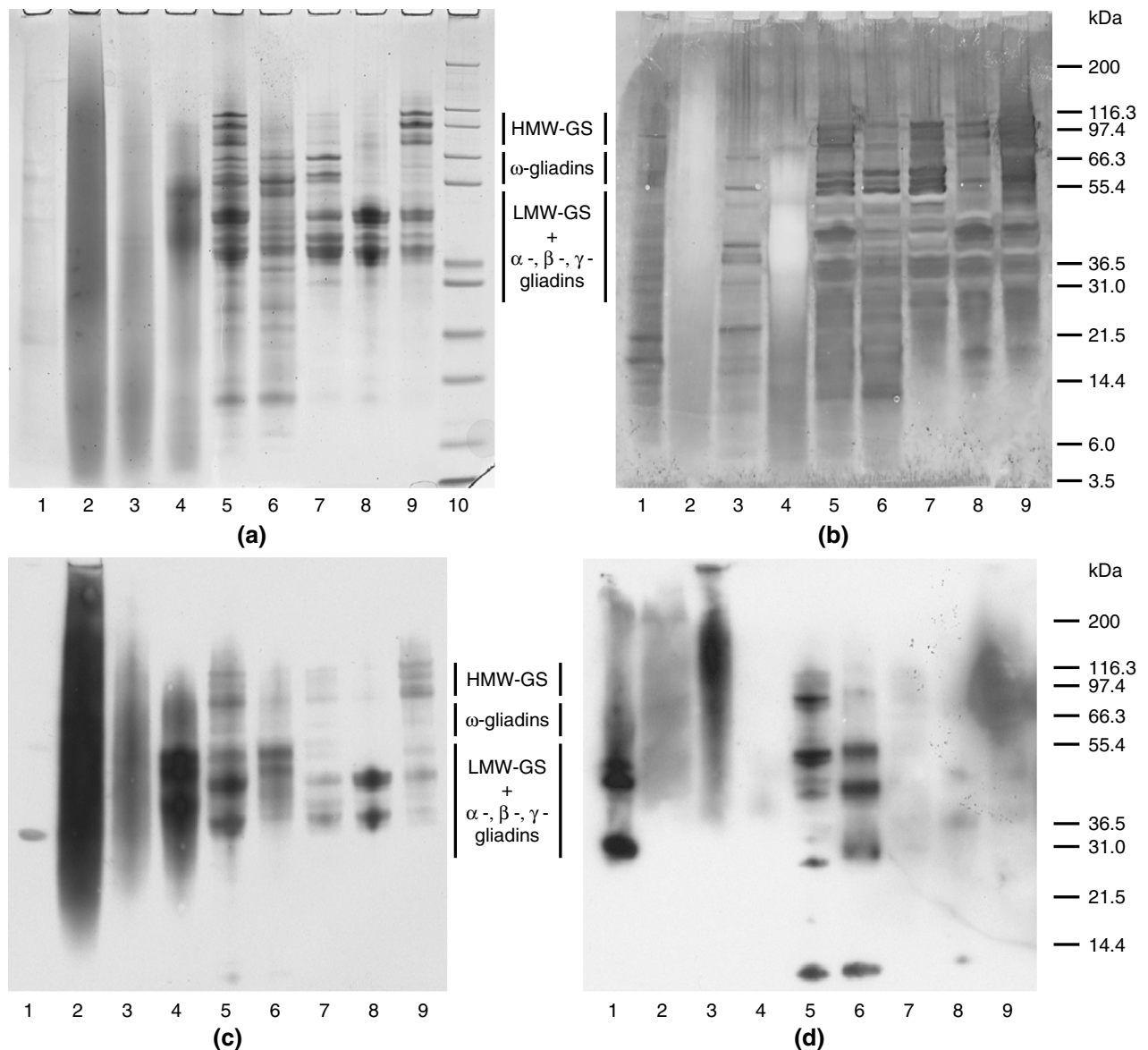


Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotting analyses of wheat protein fractions and commercial hydrolysates. (a) Gel stained with Coomassie Blue; (b) blot stained with Indian ink; (c and d) IgE reactivity from patients 1 and 4 (with and without associated food allergy), using chemiluminescence. Samples 1–3, wheat protein hydrolysates A, B and C; 4, commercial gluten; 5–9, wheat grain protein fractions, successively: 5, total proteins; 6, salt soluble proteins (albumins and globulins); 7, gliadins; 8, LMW-GS; 9, HMW-GS and LMW-GS; 10, molecular weight protein markers.

Table 3. Reactivity of patient IgE with hydrolysed gluten and with protein fractions of the wheat flour

Patient number	React to food + HWP	Hydrolysated gluten			Normal gluten	Wheat grain protein fractions				
		A	B	C		Total protein	Alb-Glob	Gliadins	LMW-GS	HMW-GS
1	+	0	++	+	++	++	++	++	++	+
2	+	++	++	+	++	+	+	+	+	+
3	+	0	++	+	++	++	++	+	0	+
4	0	++	+	++	0	++	++	0	+	+
5	+	+	++	+	++	++	++	+	++	+
6	+	ND	ND	ND	++	++	++	++	++	0
7	+	ND	ND	ND	ND	+	+	0	0	0
8	0	+	++	0	+	++	++	0	++	0
9	0	++	0	0	+	++	++	0	+	0
Dominant pattern		Ind. band	Smear	Smear	Smear	Ind. band	Ind. band	Ind. band	Ind. band	Ind. band

The dominant aspect of the IgE signal observed for the majority of patients is indicated, Ind. band, individualized band(s); smear, long smear generally from 25 kDa to the top of the gel for strong signals. Symbols 0, + and ++, respectively, indicate absent, faint and strong signals; ND, not determined with these specific samples.

Reactivity of IgE from patient's sera

All patients' IgE reacted with several protein hydrolysates (Fig. 1, lanes 1–3; Table 3). When it reacted, hydrolysate A always showed 1 or 2 individualized bands between 30 and 60 kDa, while B and C generally showed smears between 20 and 200 kDa, but sometimes much higher, up to the top of the gradient gel (Fig. 1, panels c and d). These last values largely exceeded the masses of native proteins present in gluten, which was indicative of large IgE reactive polypeptide aggregates.

Additional commercial samples from producer D, prepared by acid or enzymatic hydrolysis of the same gluten preparation, were tested with sera from patients 5 and 6. Their SDS-PAGE pattern and their reactivity with IgE were similar to those of hydrolysates B and C (Fig. 2). No noticeable difference of reactivity with IgE could be observed between the acid or enzymatic hydrolysed samples. It is remarkable that all patients' IgE also reacted with unmodified flour proteins from different fractions. Reactions were always positive with some albumins and globulins and thus with the total protein fraction. They were variable with the storage proteins: gliadins, LMW-GS and HMW-GS (Fig. 1, lanes 5–9; Table 3). Interestingly, no reaction with gliadins was observed in patients without associated food allergy. Conversely, most patients with associated food allergy reacted with gliadins. Patient 7 was the exception. Gliadins that reacted in these last cases were α/β -, γ - and ω -gliadins.

Discussion and Conclusion

All patients described in this report were primarily selected for their contact urticaria to cosmetics containing HWP and for their absence of allergy to traditional wheat products. Most of them (6/9)

did not declare any other form of allergy. The results reported here clearly showed that they reacted specifically against the HWP part of the products, through skin contact and for some of them in addition after ingestion. The skin tests made on them confirmed their tolerance to normal wheat proteins and the new allergenicity of these proteins after hydrolysis. The HWP present in the suspected products had several industrial origins. They could differ from each other by the degree and the type of hydrolysis which could be performed using acids or enzymes. The skin tests with various forms of hydrolysed proteins (i.e. the different commercial hydrolysates, and the isolated wheat protein fractions hydrolysed using acid or alkali) showed that all forms of HWP were susceptible to elicit reactions in patients.

The analysis of the IgE-reactive components present in various HWP and in unmodified wheat protein fractions showed that numerous IgE epitopes were already present in native grain proteins. They were spread on the soluble albumins and globulins but also to a variable extent on gliadins and glutenin subunits. Interestingly, IgE from patients without associated food allergy did not react with gliadins while all those with associated food allergy to product with HWP, but one, reacted with gliadins. More observations will be necessary to generalize this rule. Gliadins that reacted in these last cases belong to all groups of gliadins, unlike classical food allergy induced by effort in which $\omega 5$ -gliadin is the major allergen (6).

The observation of the IgE epitopes on native proteins contrasted with the tolerance to normal flour and traditional baked products by the patients studied. Allergic reactions occurred only when wheat proteins were partially hydrolysed. The analysis of HWP samples showed that the original electrophoretic patterns of proteins and

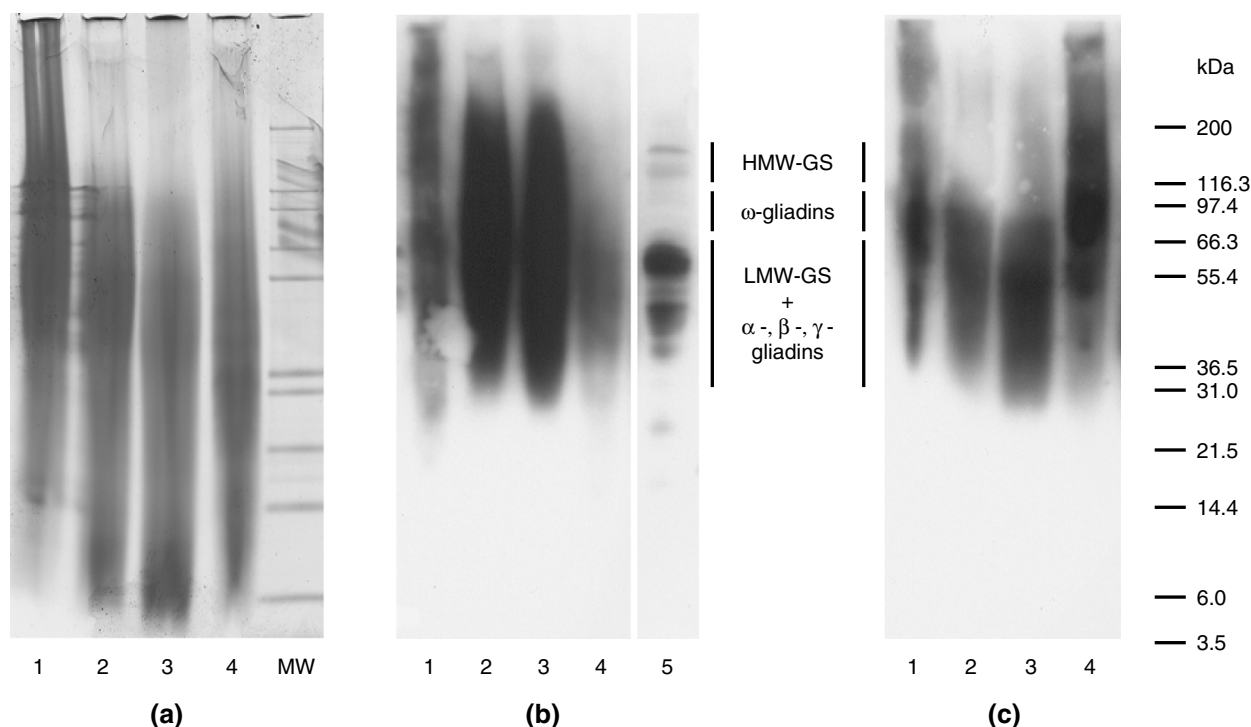


Fig. 2. Immunoblotting analyses of acidic and enzymatically hydrolysed gluten. (a) Gel stained with Coomassie Blue; (b and c) immunoblotting analysis, with patient sera 5 and 6. Samples: 1, commercial gluten; 2 and 3, two different degrees of acid hydrolysis of sample 1; 4, enzymatic hydrolysis of sample 1; 5, total wheat proteins.

of IgE-reactive proteins were fully modified. Large undefined aggregates making smears were observed, instead of the expected disappearance of large polypeptides. They looked like random rearrangements of peptides, leading to entities of molecular weight intermediate or larger than that of natural proteins. IgE reacted mostly with large aggregates. This suggested that hydrolyses did not destroy the pre-existing epitopes and the creation of multiepitopic entities, suitable for the bridging of IgE on mastocytes and basophiles. The resistance to hydrolysis of wheat protein epitopes has already been observed (10, 11). Furthermore, in the case of wheat-dependent exercise-induced anaphylaxis, Palosuo and co-workers observed that artificial polymerization of hydrolysed [omega]5-gliadin increased its direct reactivity with IgE, using immunosorbent assay, and its skin reactivity in patients, using prick tests (10). They hypothesized that large polymers had better IgE-bridging capacities. Along with the pre-existing epitopes, new epitopes are also possibly created by hydrolysis through deamidation as suggested by Leduc and co-workers (5), in the cases of acid processes, but also through the creation of normal new terminal NH_3^+ and COO^- charged groups due to the hydrolysis of peptidic bonds. All these charges increase the solubility and the possibilities of interactions with the environment of gluten polypeptides and their aggregates. This must

facilitate their transport through natural barriers. The facilitation of the transportation through the skin of these molecules is necessary to allow allergic reactions to occur. Large molecules like proteins are generally considered to do not cross the skin barrier, unless skin has been damaged (12).

The present observations point out the allergenic properties of HWP for some individuals. Their widespread uses in food and non-food products multiply the risks of sensitization. The understanding of their new allergenicity needs further studies. The fact that contact urticaria always preceded food allergy emphasizes the role of the route of contact. The occurrence of soluble, charged aggregates and their skin application were probably important factors in the observed sensitizations.

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