

The histamine content of allergen extracts

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The histamine content of 108 inhalant, food, and venom extracts from four commercial sources was measured by chemical (glass fiber-based) and immunologic (competitive RIA) methods. Histamine was present in 64 of 76 inhalant extracts (range, 0.005 to 7.4 µg/ml), 20 of 26 food extracts (range, 0.16 to 23 µg/ml), and six of six venoms, 100 µg/ml (range, 1.0 to 38 µg/ml). Histamine was removed by treatment with diamine oxidase or dialysis of an extract. Repeat assay of selected extracts after addition of known amounts of histamine in the glass fiber-based method produced additive results, and glycerin- or phenol-extract preservatives did not affect assay performance. Timed extractions of dried-pollen grains demonstrated maximal histamine content at 30 seconds, suggesting that histamine is an inherent component of at least some pollens. Histamine found in some allergen extracts could, under extreme circumstances, produce false-positive results in skin testing and in basophil histamine release assays, and could affect the result of research that uses intact pollen or allergen extracts. (J ALLERGY CLIN IMMUNOL 1992;89:738-45.)

Key words: Allergens, drug contamination, tissue extracts, pollen, skin tests, basophil histamine release, histamine, diamine oxidase

Allergen extracts are complex heterogeneous mixtures of largely unidentified biologic substances. These substances include proteins, in part allergenic, and other smaller chemical compounds characteristic of their source. Identified non-antigenic components of extracts include lectins,¹ endotoxins,^{2,3} enzymes,^{4,5} polyphenolic compounds,⁶ and hemolysins.⁷ Histamine has been detected in pokeweed mitogen.⁸ It has also been detected in Hymenoptera venoms⁹ and in house dust (0.1 µg/ml in a 1:1000 wt/vol extract)² but has not been reported as a constituent of other extracts used for allergy testing.

When basophil histamine-release tests were being performed with various allergen extracts, it was noted that false-positive results were sometimes obtained when concentrated extracts were used. This study was designed to determine whether this observation might

Abbreviations used

AO:	Allergy Laboratories of Ohio
GR:	Greer Laboratories
HS:	Hollister-Stier Laboratories
ALK:	ALK America
GFA:	Glass fiber-based histamine assay
Histamine 2 HCl:	Histamine dihydrochloride
AMAC:	Histamine RIA

be due to histamine present in the extracts. Additional experiments were undertaken to measure levels of histamine and to investigate its source.

MATERIAL AND METHODS

Commercial allergen extracts

Allergen extracts (Tables I to IV) were obtained from four commercial sources (AO, Columbus, Ohio; GR, Lenoir, N.C.; HS, Spokane, Wash.; and ALK, Milford, Conn.). Extracts were supplied in 50% glycerosaline, saline with 0.4% phenol or lyophilized. Most extracts were not standardized.

Histamine assays

The GFA was performed as previously described in detail.¹⁰ Briefly, duplicate 25 µl aliquots of the allergen extract

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were incubated with 25 μ l of buffer for 60 minutes at 37° C in glass fiber-coated microtiter wells, which selectively bind histamine.¹¹ Each extract was tested in nine 3.5-fold dilutions. To remove extract and possible nonhistamine-extract constituents, incubation was followed by an enzymatic rinse and washing of the microtiter plates in deionized water.¹⁰ Histamine was dissociated from the glass fibers by adding a 2% o-phthaldialdehyde-NaOH solution; this solution-histamine complex was stabilized with 0.59% HClO₄, and fluorescence values were determined in a photofluorometer (Gilson Medical Electronics, Middleton, Wis.). Results are expressed in nanograms per milliliter of histamine 2 HCl by use of a reference curve. Reported results were within the linear range of the assay (1 to 150 ng/ml of histamine 2 HCl).

Histamine RIA (AMAC) was performed with a commercially available kit (AMAC Inc., Immunotech S.A., Marseille, France).^{12, 13} Briefly, 100 μ l of various dilutions of each extract were acylated and placed in competition overnight at 4° C with a radioiodinated acylated histamine tracer in tubes coated with monoclonal antibody against acylated histamine. Tubes were aspirated, and bound radioactivity was measured in a gamma counter. Assays were performed in duplicate. Results in nanomoles of histamine were calculated by cubic spline interpolation from a six-point reference curve, corrected for dilution, and converted to nanograms per milliliter of histamine 2 HCl by multiplying by 0.184. Reported assay results were in the linear range of the assay (0.18 to 3.7 ng/ml of histamine 2 HCl).

Analytic specificity

The analytic performance of both histamine assays has previously been examined extensively with respect to specificity, sensitivity, and reproducibility.^{11, 13} The assays were found to be precise and demonstrated a high degree of correlation.

To verify presence of histamine, dilutions of box elder (HS), Johnson-grass (GR), and short ragweed (GR) extracts were incubated with and without 1 U/ml of diamine oxidase (Sigma Chemical Co., St. Louis, Mo.) for 1 hour at 37° C. This procedure was performed after the enzymatic rinse step in the GFA method.

An undiluted Johnson-grass extract (GR) was dialyzed against 200 vol of distilled water overnight at room temperature with a 1000 molecular weight cutoff membrane (Spectrapor, Spectrum Medical Industries, Inc., Los Angeles, Calif.). This extract was from a different lot than that reported in Table I. Assays were performed by the GFA method.

To examine a possible effect of extract constituents on the histamine measurements, dilutions of extracts of Bahia grass, short ragweed, and box elder, which had low, moderate, and high histamine content, were measured in GFA wells preloaded with 25 and 50 ng of histamine 2 HCl (Sigma Chemical Co.). Additionally, the effect of glycerin (0%, 0.7%, 1.5%, 3.2%, 6.3%, 12.5%, and 25%) on measurement of various concentrations of histamine 2 HCl (0, 25, 50, 75, and 100 ng/ml) in saline was evaluated by GFA. The effect of glycerin (0%, 0.8%, 1.6%, 3.2%, 6.3%, and

12.5%) and phenol (0%, 0.6%, 0.12%, 0.25%, and 0.5%) on the measurement of histamine 2 HCl (0, 2.3, and 7.1 ng/ml) in saline was studied by AMAC.

Pollen extractions

Dried Johnson-grass pollen (HS) was extracted at 1:10 wt/vol in 0.85% saline with and without 0.4% phenol or 50% glycerinated saline without phenol at 4° and 37° C. Extraction times ranged from 10 minutes to 48 hours. All extracts were made in polypropylene tubes (Stockwell Scientific, Walnut, Calif.) and centrifuged at 10,000g for 10 minutes. The extracts, which were not sterilized, were stored at -20° C until assay for histamine content by GFA.

Dried box elder pollen (HS) was extracted at 1:10 wt/vol in 0.85% saline without phenol at room temperature for periods of time ranging from 30 seconds to 240 minutes. Extracts were immediately filter sterilized with a 0.2 μ m filter (Gelman Sciences, Ann Arbor, Mich.), stored at -20° C, and analyzed by both histamine assays. In all experiments, 0.85% saline aliquots treated in the same manner served as controls.

RESULTS

Histamine content of extracts

Nearly all the allergen extracts examined contained measurable amounts of histamine. Histamine was present in 48 of 49 pollen extracts, ranging from 0.005 to 7.4 μ g/ml (Table I). Of these pollen extracts, box elder, short ragweed, and Johnson grass had the highest histamine content. Twenty of the 26 food extracts examined contained histamine, ranging from 0.16 to 23 μ g/ml (Table II). The extracts of saltwater fish, tomato, and casein contained the largest amounts of histamine. Histamine was detected in 12 of 14 mold extracts; levels were low with a range from 0.02 to 0.88 μ g/ml (Table III). The epidermal extracts, insect extracts, venoms, and miscellaneous extracts ranged from 0 to 38 μ g/ml (Table IV). The insect venoms and cat-hair extracts contained the highest amounts of histamine. No histamine was found in a standardized extract of cat epithelium (ALK) which had been dialyzed during manufacture. Different sources and lots of the same extract varied in histamine content.

Analytic specificity

The AMAC assay was not performed on every extract. When an assay was done, results agreed well ($n = 40$; $r_s = 0.85$; $p < 0.001$; Spearman's rank correlation) with GFA.

The addition of diamine oxidase during the GFA of the histamine-containing extracts resulted in a nearly complete loss of measurable histamine (Table V). For simplicity, results of experiments with 1:42.9 vol/vol (box elder and Johnson grass) and 1:3.5 vol/vol (short ragweed) dilutions are presented. The histamine mea-

TABLE I. Histamine 2 HCl content of 49 commercially available pollen-allergen extracts

Pollen extract	Concentration*	Histamine	
		GFA ($\mu\text{g/ml}$)	AMAC ($\mu\text{g/ml}$)
Box elder (AO)	1:20	7.4	7.2
Box elder (HS)	1:20	5.6	6.8
Short ragweed (GR)	1:10	2.6	
Johnson grass (GR)	1:10	2.4	
Southern grass mix (GR)	1:10	1.5	
Lamb's-quarter (AO)	1:20	1.4	
Dandelion (AO)	1:20	0.75	
Short ragweed (HS)	1:20	0.68	0.57
Johnson grass (AO)	1:20	0.62	
Russian thistle (AO)	1:20	0.60	
Cottonwood (HS)	1:20	0.53	0.49
Short ragweed (HS)	1:20	0.38	0.42
Timothy (HS)	1:20	0.28	0.25
Common sage (HS)	1:20	0.24	
Russian thistle (HS)	1:20	0.18	0.20
Chinese elm (AO)	1:20	0.17	0.22
Siberian elm (HS)	1:20	0.17	0.18
Alfalfa (AO)	1:20	0.16	
Russian thistle (HS)	1:20	0.15	
Timothy grass (HS)	1:20	0.13	0.08
Bermuda (AO)	1:20	0.12	
Sagebrush (AO)	1:20	0.11	0.15
Spring birch (HS)	1:20	0.11	0.18
Cottonwood (AO)	1:20	0.10	0.65
Blue grass (HS)	1:20	0.097	0.12
June grass (AO)	1:20	0.094	
English plantain (HS)	1:20	0.088	0.064
Short ragweed (AO)	1:20	0.088	
Meadow fescue (AO)	1:20	0.088	
Meadow fescue (HS)	1:20	0.078	0.065
Shadscale (AO)	1:20	0.078	
Brome grass (AO)	1:20	0.069	
English plantain (HS)	1:20	0.065	0.067
Black locust (AO)	1:20	0.060	
Timothy (AO)	1:20	0.058	
Bermuda grass (HS)	1:20	0.058	0.045
Burr oak (AO)	1:20	0.056	
Goldenrod (AO)	1:20	0.055	
Blue grass (HS)	1:20	0.055	0.032
Mountain cedar (GR)	1:20	0.039	
English plantain (AO)	1:20	0.039	0.025
White ash (AO)	1:20	0.036	
Spring birch (HS)	1:20	0.025	0.015
Pigweed (HS)	1:20	0.024	0.032
Kochia (AO)	1:20	0.021	
Russian olive (AO)	1:40	0.0075	
River birch (AO)	1:20	0.0050	0.029
Mountain cedar (HS)	1:20	0.0050	0.0010
Ponderosa pine (AO)	1:20	0	

Extractions are ranked in order of decreasing histamine content.

*Weight per volume unless it is otherwise specified.

TABLE II. Histamine 2 HCl content of 26 commercially available food-allergen extracts

Food extract	Concentration*	Histamine	
		GFA ($\mu\text{g/ml}$)	AMAC ($\mu\text{g/ml}$)
Mackerel (GR)	1:10	23	>23
Sole (AO)	1:10	18	
Fish mix (GR)	1:10	9.0	
Tomato (GR)	1:10	5.0	
Casein (GR)	1:40	1.1	
Salmon (AO)	1:10	0.90	
Beef (GR)	1:10	0.79	
Pork (AO)	1:10	0.53	
Egg white (AO)	1:10	0.43	
Oyster (AO)	1:10	0.30	
Clam (AO)	1:10	0.26	
Cow's milk, whole (AO)	1:10	0.22	
Lobster (AO)	1:10	0.19	
Trout (GR)	1:20	0.17	
Shrimp (AO)	1:10	0.16	
Crab (AO)	1:10	0.15	
Wheat (AO)	1:10	0.70	
Codfish (AO)	1:10	0.67	
Soybean (AO)	1:10	0.37	
Corn (AO)	1:10	0.16	
Whole egg (GR)	1:10	0	
Rye (GR)	1:10	0	
Peanut (AO)	1:10	0	
English walnut (GR)	1:10	0	
Filbert (GR)	1:10	0	
Soybean (GR)	1:10	0	

Extracts are ranked in order of decreasing, histamine content.

*Weight per volume unless it is otherwise specified.

TABLE III. Histamine 2 HCl content of 14 commercially available mold-allergen extracts

Mold extracts	Concentration*	Histamine	
		GFA ($\mu\text{g/ml}$)	AMAC ($\mu\text{g/ml}$)
<i>Candida albicans</i> (AO)	1:20	0.88	
<i>Cladosporium</i> (HS)	1:10	0.29	0.26
<i>Alternaria</i> (GR)	1:10	0.27	0.26
<i>Alternaria</i> (HS)	1:10	0.23	0.33
<i>Aspergillus fumigatus</i> (HS)	1:10	0.20	0.25
<i>Epicoccum</i> (AO)	1:20	0.12	
<i>Rhizopus</i> (AO)	1:20	0.12	
<i>Cladosporium</i> (AO)	1:20	0.064	0.090
<i>Aspergillus niger</i> (AO)	1:20	0.059	
<i>Alternaria</i> (AO)	1:20	0.020	
<i>Aspergillus fumigatus</i> (AO)	1:20	0.020	0.029
<i>Penicillium</i> (AO)	1:20	0.020	
<i>Alternaria</i> (GR)	1:10	0	
<i>Stemphylium</i> (AO)	1:20	0	

Extracts are ranked in order of decreasing histamine content.

*Weight per volume unless it is otherwise specified.

TABLE IV. Histamine 2 HCl content of 17 other commercially available allergen extracts

Extract	Concentration*	Histamine	
		GFA (μg/ml)	AMAC (μg/ml)
Mixed vespid (ALK)	300 μg/ml	37.8	
White-faced hornet venom (HS)	100 μg/ml	11	9.8
Yellow jacket venom (HS)	100 μg/ml	9.5	7.7
Yellow hornet venom (HS)	100 μg/ml	5.5	4.3
Honeybee venom (HS)	100 μg/ml	4.5	4.4
AP cat-hair epithelium (HS)	1:50	2.4	1.5
Honey bee venom (ALK)	100 μg/ml	1.03	
AP cat hair (HS)	1:50	0.59	0.70
<i>Dermatophagoides pteronyssinus</i> (HS)	10,000 AU/ml	0:20	0.14
Cat-hair epithelium (HS)	1:10	0.19	0.16
Cockroach (AO)	1:20	0	
Kapok (AO)	1:20	0	
Orris root (AO)	1:20	0	
House dust (AO)	1:20	0	
House dust mix (HS)	1:10	0	0.02
<i>D. farinae</i> (HS)	10,000 AU/ml	0	0
Dog-hair dander (HS)	1:10	0	
<i>D. farinae</i> (AO)	1:50	0	
Cat (AL)	100,000 AU/ml	0	

AP, Acetone precipitated.

Extracts are ranked in order of decreasing histamine content.

*Weight per volume unless it is otherwise specified.

TABLE V. Analytic specificity of histamine assays

Extract	Dilution assayed (vol/vol)	Histamine (before)*	Histamine (after)*	% Reduction
Digestion of histamine by diamine oxidase (1 U/ml)				
Box elder	1:43	196	19.0	90
Johnson grass	1:43	58.5	2.50	96
Short ragweed	1:3.5	93.0	12.5	87
Dialysis of histamine				
Johnson grass		3151	0	
	Histamine (before)*	Histamine added	Histamine (after)*	Difference
Assay of extracts after preloading of GFA plates with histamine				
Box elder	20.3	25 ng	57.3	37.0
(1:150 vol/vol)		50 ng	68.8	48.5
Bahia grass	30.5	25 ng	57.0	26.5
(1:12 vol/vol)		50 ng	86.5	56.0
Short ragweed	32.5	25 ng	56.5	23.9
(1:43 vol/vol)		50 ng	75.0	42.5

*Reported as nanograms per milliliter of histamine 2 HCl by GFA method.

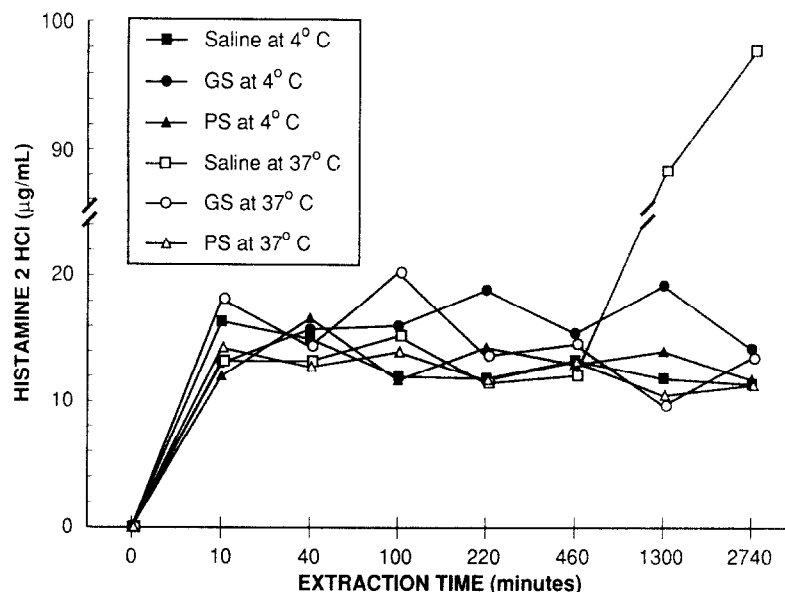


FIG. 1. Timed extractions of Johnson-grass pollen, assayed for histamine content by GFA method. Histamine content was maximal at 10 minutes, first point assayed. Extract made in preservative-free saline at 37° C produced high levels of histamine after 22 hours of extraction. This cloudy, malodorous extract may have been contaminated; GS, glycerinated saline; PS, phenolated saline.

sured in a Johnson-grass extract was completely removed by dialysis (Table V).

Assay of dilutions of extracts of box elder, short ragweed, and Bahia grass after preloading of GFA wells with 25 and 50 ng/ml of histamine 2 HCl demonstrated an additive increase in histamine content, suggesting that no substances in the extract interfered with the recovery of added histamine (Table V). Results of experiments with 1:150 vol/vol (box elder), 1:42.9 vol/vol (short ragweed), and 1:12.2 vol/vol (Bahia grass) are presented. No concentration of glycerin tested interfered with either assay. Phenol, in the concentrations tested, did not interfere with the AMAC histamine assay.

Source of histamine in pollen extracts

Histamine in the Johnson-grass extract was detected very early in the extraction procedure, achieving maximum levels on an average of 17.7 µg/ml within 10 minutes, the first time point assayed (Fig. 1). This finding was independent of temperature and the presence of 50% glycerin or 0.4% phenol. Levels were approximately fivefold to 30-fold higher than levels observed in different commercial extracts of this pollen. One Johnson-grass extract prepared in saline at 37° C without preservatives demonstrated a very high level of histamine (88.5 µg/ml) after 22 hours of extraction. The extract was cloudy and malodorous and was not cultured.

The box elder extract demonstrated maximum his-

tamine content (20 to 26 µg/ml) after 30 seconds of extraction, again the first time point assayed (Fig. 2). This level was two to four times higher than that of box elder extracts obtained from commercial sources.

DISCUSSION

Histamine is a known constituent of insect venoms⁹ and certain foods, in some cases produced by the action of bacteria that decarboxylate the amino acid, histidine.^{14, 15} Finding histamine in many commercially available allergen extracts was not expected. Both assays are highly specific for histamine, and results of the two different assays agreed. The histamine could be removed from extracts by diamine oxidase and dialysis. Preloading of GFA plates with histamine produced additive results, and no influence of inert extract constituents on the assays was demonstrable. These observations indicate that the substance measured is actually histamine, rather than an interfering or cross-reacting factor.

Histamine could be present in the original source material, could be produced during the extraction procedures, or both. The study indicates that histamine is inherently present in Johnson-grass and box elder pollen because of rapid and complete release in extraction. Furthermore, if this were enzymatically manufactured histamine, temperature and protein denaturing substances, such as glycerin and phenol, would be expected to affect the process substantially. This was not observed. These extracts, prepared in poly-

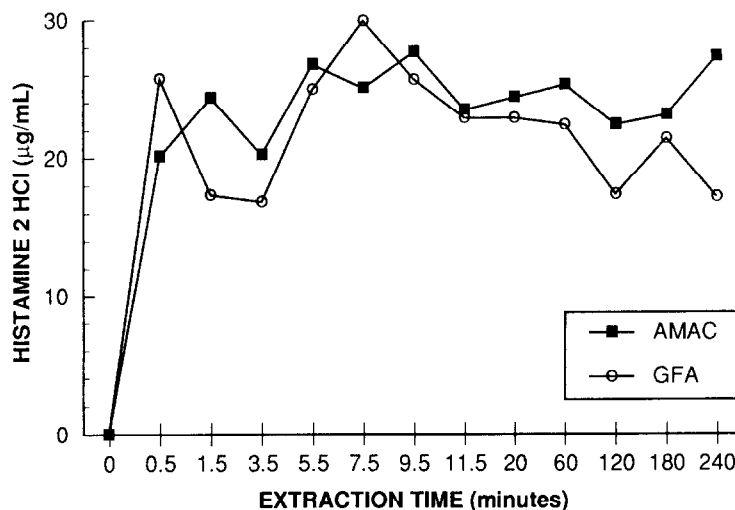


FIG. 2. Timed extraction of box elder pollen, assayed for histamine by AMAC and GFA methods. Histamine content was maximal at 30 seconds, first point assayed.

styrene vessels, contained five to 30 and two to four times more histamine (Johnson grass and box elder, respectively) as their commercial counterparts. Since histamine specifically binds to glass surfaces,¹⁶ commercial extraction procedures that use glass equipment for production of allergen extracts would be expected to decrease the amount of histamine in the final product. In contrast, apparent bacterial contamination of the Johnson-grass extract made without preservatives or sterilization may have produced extremely high histamine levels.

The clinical implication of use of extracts containing histamine for skin testing warrants comment. Studies of the relationship between histamine concentration and skin test response have generally ignored small reactions because skin testing loses precision when wheal areas are small¹⁷ and because such studies focus on reactivity in groups of patients rather than in individuals. It is thus difficult to determine, in a worst case scenario, what is the lowest concentration of histamine that would produce a visible skin test reaction in an individual highly reactive to histamine.

In epicutaneous skin testing, when wheal areas $>7 \text{ mm}^2$ (3 mm in diameter) were considered positive, Dreborg et al.¹⁸ did not find skin reactivity to 160 $\mu\text{g/ml}$ or lower concentrations of histamine 2 HCl in 20 symptomatic allergic patients. With prick skin testing at a concentration of 49 $\mu\text{g/ml}$ of histamine 2 HCl, Harris et al.¹⁷ reported mean wheal area of about 2 mm^2 with a standard deviation of about 2.5 mm^2 in 26 symptomatic allergic patients. Malling,¹⁹ however, calculated from dose-response curves, that in prick skin testing the histamine 2 HCl concentration

required to elicit a wheal area of 2 mm^2 in asymptomatic subjects with negative skin tests ($N = 7$), asymptomatic but sensitized individuals ($N = 7$), and symptomatic allergic patients ($N = 20$) would be 530, 150, and 6 $\mu\text{g/ml}$, respectively. Four of the extracts assayed (box elder, mackerel, sole, and fish mix) contained $>6 \mu\text{g/ml}$ of histamine in concentrations used for prick skin testing. These extracts might contain enough histamine to produce visible wheal reactions, which might be interpreted by some clinicians as positive²⁰ in a few patients.

In considering intracutaneous skin testing, it is unclear whether these and the other extracts with lower amounts of histamine might produce false-positive results in some individuals if the customary 1:500 to 1:1000 wt/vol concentrations²¹ and conservative interpretation criteria (e.g., wheal diameter $>10 \text{ mm}$ considered positive) are used. In intradermal testing, Turkeltaub et al.²² reported a single patient in whom the sum of erythema ranged from about 15 to 70 mm with concentrations of histamine ranging from about 0.015 to 1.4 $\mu\text{g/ml}$ of histamine base (about 0.024 to 2.2 $\mu\text{g/ml}$ of histamine 2 HCl); wheal measurements were not reported. Djukanovic et al.²³ reported small wheal-and-flare responses in intradermal testing of atopic and nonatopic individuals with a concentration as low as 0.2 $\mu\text{g/ml}$ of histamine phosphate (about 0.17 $\mu\text{g/ml}$ of histamine 2 HCl), the lowest concentration tested. When more concentrated extracts²⁴ or liberal cutoff criteria (wheal, 3 to 4 mm larger than control wheal)^{20, 24} are used in intracutaneous testing, more false-positive results might be expected. This finding could, in part, explain previous observations that the apparent false-positive rate in

intracutaneous skin testing increases as more concentrated extracts are used.²⁵

Factors, such as testing method, individual patient differences, choice of skin testing site, lot-to-lot extract variation in histamine content, extract storage conditions, extract concentration used for testing, criteria for a positive result, and testing device used, could act separately or in combination to produce false-positive skin test results. The clinical relevance of this one factor, the finding of histamine in allergen extracts, and the influence of various pollen extraction procedures and lot-to-lot variation on histamine content warrant detailed study. The histamine content of a new extract lot should routinely be measured by the manufacturer.

In research applications with intact pollen grains, extracts made on premises or commercially available extracts, histamine content of the material should be taken into account to determine whether any histamine present could affect study outcome. This determination is particularly important in studies of the biologic potency of extracts, since skin test sensitivity could be influenced by histamine present in the extract as well as histamine released from mast cells. Extracts to be used in basophil histamine-release testing should be assayed for histamine content, particularly if concentrated extracts are used. Furthermore, lyophilized extracts should be reconstituted in a diluent containing preservative or used immediately after reconstitution.

Note added in proof. Histamine has been found in stored pollen (Marquardt P, Vogg G. *Pharmakologische und chemische Untersuchungen iibes Wirkstoffe in Bienenpollen*. *Arzneimittelforschung* 1952;2: 267-71.).

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