

Non-Allergenic Haemolysins in Grass Pollens and Housedust Mites

D. L. J. FREED, C. H. BUCKLEY, Y. TSIVION, N. SHARON and D. H. KATZ

Dept. of Bacteriology & Virology and Dept. of Pathology, University of Manchester, England,
Dept. of Biophysics, Weizmann Institute, Rehovoth, Israel, and Dept. of Molecular Immunology,
Scripps Clinic and Research Foundation, La Jolla, California, U.S.A.

25 batches of pollen (six common grasses, maize, short ragweed) and two batches of housedust mite (*Dermatophagoides pteronyssinus*) all contained haemolysins. The haemolysins of two grass pollens and of the housedust mites were of small MW (100-200 Da) and apparently non-allergenic. Both caused inflammation when injected into human skin, at doses that could be experienced naturally. The "allergic" airways diseases may not be entirely immunologically mediated.

Key words: allergens; haemolysins; hay fever; mites; pollinosis; toxins; Type I hypersensitivity.

Accepted for publication 2 April 1983

Hay fever is caused by a Type I allergic response (1) to inhaled pollen grains. The reaction is mediated by antibodies, largely of the IgE class, bound to mast cells in the respiratory and ocular membranes, which on exposure to the allergen liberate pharmacologic mediators such as histamine. Before this mechanism was understood, it was generally supposed that hay fever is caused by a susceptibility of certain people to poisons in pollen (2). Although we now understand the role of allergy, the earlier notion might be true as well. We here report that grass pollens and housedust mites – numerically the greatest cause of Type I respiratory allergy worldwide – do indeed contain toxic molecules, which are non-allergenic but may nonetheless contribute to morbidity.

MATERIAL AND METHODS

Allergenic materials

Dry pollens of six common British grasses (*Lolium perenne*, *Holcus lanata*, *Poa pratensis*, *Dactylis glomerata*, *Phleum pratense* and *Festuca pratensis*) and of short ragweed (*Ambrosia eliator*) were

obtained from E. Merck Ltd., UK; Allergon AB, Sweden; Miles Laboratories Ltd., UK; and Greer Laboratories, USA. Dried killed housedust mites (*Dermatophagoides pteronyssinus*) were donated by E. Merck Ltd., UK. Fresh maize (*Zea mays*) pollen was collected by Y.T.

Extracts of pollen were made by shaking in distilled water (2% w/v) at room temperature for 5 min, then clarifying by bench centrifugation and/or paper filtration. Solutions to be tested for toxicity were brought to physiological pH and osmolality with barbitone buffer (Oxoid) and 1.0 N NaOH, and sterilized by "Millipore" filtration or autoclave (121°C, 15 min). Solutions to be fractionated were concentrated by heat (60-100°C) under a stream of nitrogen. Housedust mites were extracted in the same way except that a 15% (w/v) mixture was used for the initial extraction. Since the extracts were not preserved chemically, they were always tested on the day of preparation.

Weight of pollen grains

This was measured by weighing a small amount of dry pollen, then suspending it in butanol and

diluting until individual grains could be counted microscopically.

Toxicity testing in vitro

Cunningham chambers (3) were made by fixing clean dry microscope slides face-to-face with double sided sticky tape. Mixtures of pollen grains and 5% sheep erythrocyte suspension in barbitone buffer pH 7.2 (4) were allowed to flow into the gap by capillarity and the edges were sealed with wax. These preparations were incubated at 37°C overnight.

Doubling dilutions of pollen and housedust extracts (200 µl) were dispensed into the wells of perspex WHO haemagglutination trays, using either barbitone buffer, or phosphate buffer (pH 7.2; 0.07 M) which had been supplemented with 100 mg/l each of CaCl₂ and MgCl₂ (un-supplemented phosphate buffer inhibited haemolysis). A 5% suspension of erythrocytes (100 µl) was added to each well and the plates incubated at 37°C overnight. Haemolysis was indicated by disappearance of the cell button and uniform haemoglobin staining of the solution.

Pollen extracts in "Tc199" tissue-culture medium (Flow Laboratories) were incubated with hela cell monolayers, without any serum in the medium. All incubations were at 37°C overnight. The criteria for toxicity were lysis of the erythrocytes or dehesion and trypan-blue staining of the hela cells.

Biochemical analysis of pollen haemolysins

Lolium perenne (ryegrass) and *Dactylis glomerata* (cocksfoot) pollen extracts were evaporated to dryness by heating to 60–100°C under a flow of nitrogen, or by lyophilisation. The dry residue of each was mixed with dry acetone for 15 min, and the acetone-insoluble portion (which contained most of the haemolytic activity) was re-dissolved in 10 mM ammonium acetate solution and re-clarified by centrifugation at 10,000 g. Preliminary experiments showed that the haemolytic activity was removed by dialysis and would pass through an Amicon "UMO5" ultra-filter (nominal cutoff MW = 500 Da), so the

acetone-insoluble material was fractionated on a column (2.5 cm × 45 cm) of "Biogel" P2 (Biorad Laboratories; nominal separation range about 200–2,000 Da). Fractions possessing haemolytic activity were then applied to Merck Silica Gel 60 (F-254) preparative layer chromatography plates and run in a solvent of water, acetone, methanol and ammonia in a ratio 2:3:3:1. Amino acid analysis, without preliminary hydrolysis, was performed on one of the haemolytic fractions obtained by this procedure, using a Beckman "Auto-Analyzer".

Allergenicity testing

Radioallergosorbent (RAST) tests were devised using *L. perenne* pollen or *Dermatophagoides pteronyssinus* coupled to paper discs (Pharmacia, Uxbridge, U.K.). 50 µl of pooled atopic serum (from 6 hay-fever patients), or serum from D.L.J.F., was added to the disc together with 50 µl of either buffer or test extract. After incubation the discs were washed and reincubated with 50 µl ¹²⁵I-labelled anti-human IgE, then washed, and bound radioactivity was counted. Allergenicity was also tested *in vivo* by prick testing (5) in seven atopic volunteers.

Toxicity testing in vivo

Thirteen healthy medical students and two of us (D.L.J.F. and Y.T.) volunteered after full discussion. Six volunteers were hay fever sufferers, three had type I syndromes but not to pollen, and six had no allergic symptoms past or present either in themselves or their families. All were prick-tested in the conventional way (5) for skin sensitivity to mixed grass pollen and housedust mite (Bencard); the six non-allergic subjects were skin-test negative and the others were skin-test positive to both allergens (except for D.L.J.F., who is skin-test positive only to housedust mite).

Intradermal injections (0.025 ml) of allergen-free *L. perenne* haemolysin (fraction L1; see below) were administered in the forearm skin, starting at a dose of 0.1 µg in sterile physiological saline and increasing the dosage by 10-fold steps at 24-h intervals until redness and/or

Table 1

Haemolytic titres of various pollens, tested on various occasions, with and without prior autoclaving (121°C, 15 min)

Date tested	Species of pollen	By whom supplied and when	Titre of haemolysis	
			before autoclaving	after autoclaving
May 1977	<i>Phleum pratense</i>	Miles, Jan. 1977 (i.e. harvested in 1976)	256, 1024	256, 512
	<i>D. glomerata</i>		128, 512, 256, 64	512, 256
Aug. 1978	<i>F. pratensis</i>	Merck Aug. 1978	8	NT*
	<i>D. glomerata</i>		16, 256	NT
	<i>H. lanata</i>		4	NT
	<i>Poa pratensis</i>		8	NT
	<i>Phleum pratense</i>		16	NT
	<i>L. perenne</i>		16	NT
June 1979	<i>L. perenne</i>	Manchester Univ. Dept. of Botany June 1979	NT	512
Sept. 1980	<i>D. glomerata</i>	Miles June 1980	NT	16
	<i>Phleum pratense</i>		NT	8
Sept. 1980	<i>Phleum pratense</i>	Merck June 1980	NT	2, 16
	<i>D. glomerata</i>		NT	16
	<i>L. perenne</i>		NT	128, 256
Sept. 1980	<i>Zea mays</i>	Y.T. Sept. 1980	NT	64
Nov. 1981	<i>Phleum pratense</i>	Greer Sept. 1979	NT	32, 64
Nov. 1981	<i>Ambrosia eliator</i>	Greer July 1980	NT	16, 32
Oct. 1982	<i>Phleum pratense</i>	Merck May 1982	16	8
	<i>L. pratense</i>		4	16
	<i>D. glomerata</i>		8	4
Oct. 1982	<i>Phleum pratense</i>	Allergon April 1982	1	0
	<i>Phleum pratense</i>		0	1
	<i>D. glomerata</i>		2	1
	<i>L. perenne</i>		0	1
	<i>L. perenne</i>		2	2

* NT = not tested.

pain (as compared with a control injection of sterile saline) was observed at the injection site, 24 h after the injection.

Eleven of the subjects volunteered to have a biopsy taken of a representative injection site. A

small sliver (10 mm × 2 mm) of skin, full-thickness, was removed with scalpel and forceps under local anaesthesia, fixed in formalin and examined microscopically with haematoxylin and-eosin staining.

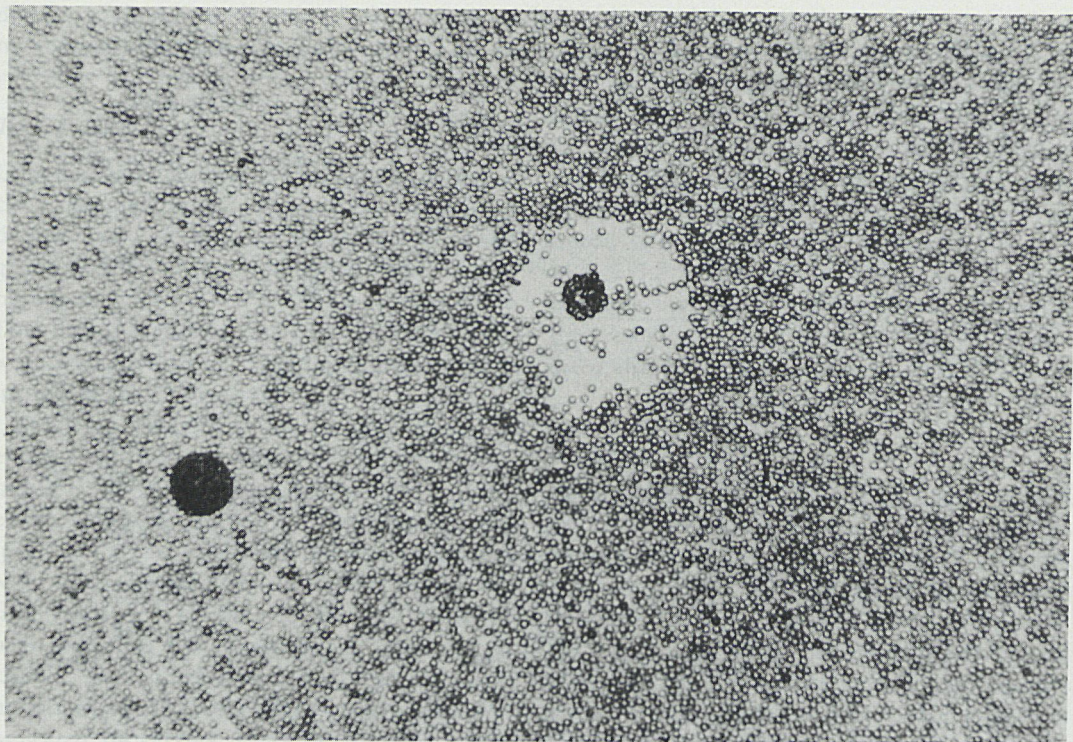


Fig. 1. Two grains of *Dactylis glomerata* pollen in Cunningham chamber, mixed with sheep erythrocytes and incubated at 37°C overnight. One grain has discharged its cytoplasm and is surrounded by a zone of haemolysis (the zone is littered with ghosts but these cannot be seen in this photograph). Magnified approx. 125 ×.

D.L.J.F., who is allergic to housedust mite, received three intradermal injections of allergen-depleted extract (see p. 484) of *D. pteronyssinus*. One was biopsied at 24 h and two after 2 weeks. One of the injections was preceded (4½ H) by an intravenous injection of 60 mg prednisolone.

RESULTS

Toxicity in vitro

All of the pollen extracts tested (8 species; 25 batches in all) were haemolytic when incubated with the erythrocytes of human, sheep, horse, ox and fowl, showing no species preference (Table 1). At high pollen concentrations the laked haemoglobin assumed a greenish tinge. The minimum haemolytic doses of two pollen extracts (one batch of *Dactylis glomerata* and one of *Phleum pratense*) were found to be 26 µg/ml

and 6 µg/ml respectively (total dry solids of extract), for pollens collected in 1976. Titres of pollen haemolysin were highest in that year (a hot dry summer in Europe) and rather lower in subsequent years. Haemolysis was occasionally absent at the higher concentrations of pollen extract, and was usually inhibited, though sometimes enhanced, by autoclaving (115°C, 15 min). Two batches were not haemolytic at all until autoclaved. Haemolysis was reduced or abolished by addition of 0.1 M EDTA, or by doing the experiment in unused, new polystyrene "Microtiter" plates, or in unsupplemented phosphate buffer.

The Cunningham chambers showed that after wetting, about 15% of the pollen grains in any one preparation had discharged their cytoplasm through a pore, as noted a century ago by Blackley (6). With two batches of pollen (one of *Dactylis glomerata*, one of *Phleum pratense*) at pollen:erythrocyte ratio = 1:14,000, zones of

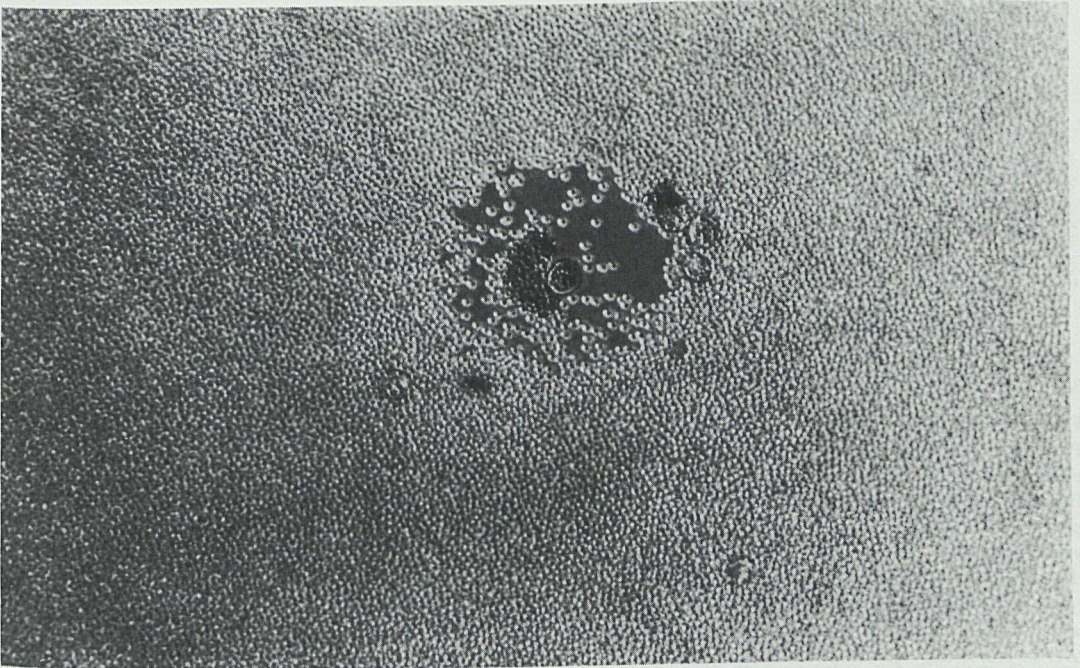


Fig. 2. Discharged grain of *Phleum pratense* pollen, with package of cytoplasm lying to its left and densely surrounded by erythrocytes. The zone surrounding is clear and does not contain ghosts. Several undischarged grains are dimly seen amid the surrounding erythrocytes. Magnification approx. 145 $\times$.

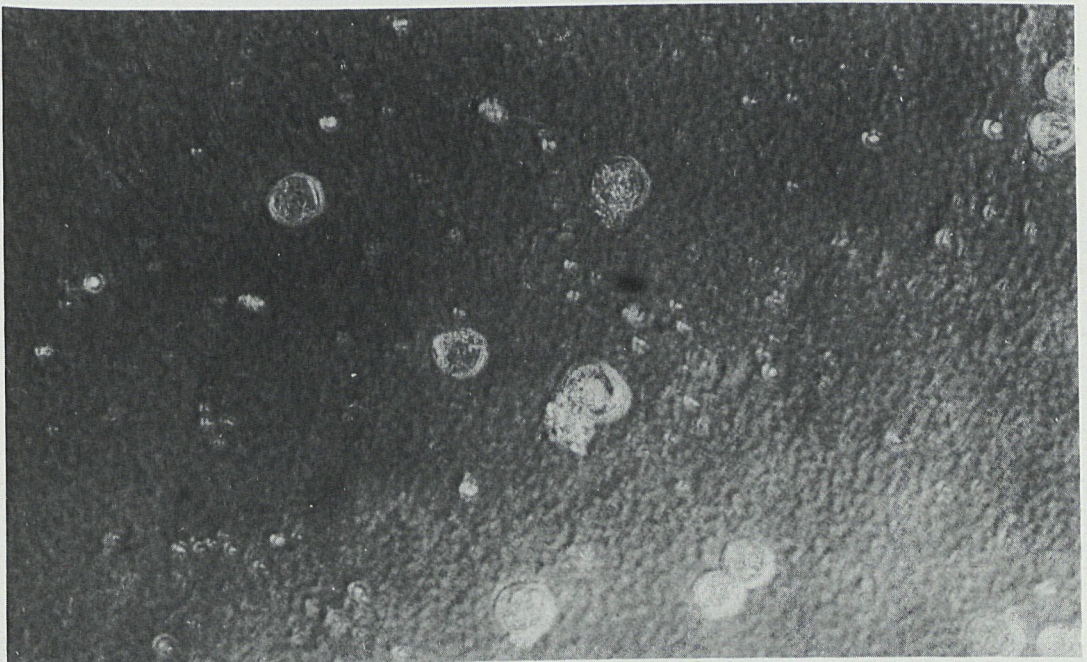


Fig. 3. Several grains of *Dactylis glomerata* pollen, at higher pollen/erythrocyte ratio (approx. 1:1400) than in Fig. 1 and 2, showing total haemolysis of surrounding erythrocyte sheet. Magnification approx. 200 $\times$.

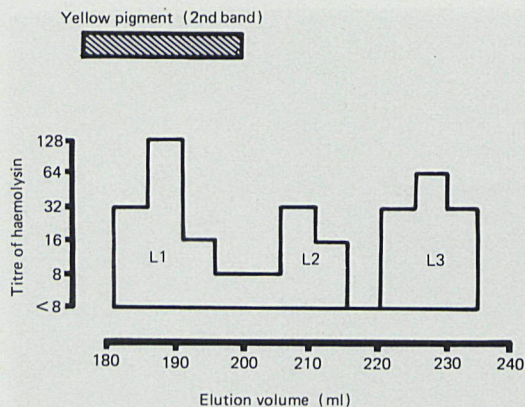


Fig. 4. Elution of *Lolium perenne* pollen extract from "Biogel" P2 column showing titre of haemolysin as a function of elution volume. The three peaks were named L1, L2 and L3 from left to right. *Dactylis glomerata* separated into three peaks of haemolysin that correspond almost exactly. The yellow pollen pigment is split by the chromatography into three bands; the first (brown-grey) eluting before the haemolysins at 87–107 ml, the second (brown-yellow) corresponding to L1, and the third (lemon yellow) eluting very slowly over several hours, well behind the haemolysins.

haemolysis were seen, littered with erythrocyte ghosts, surrounding discharged pollen grains (Fig. 1). With five other pollen batches, cleared zones were observed, due not to haemolysis but to aggregation of erythrocytes (Fig. 2). At high pollen/erythrocyte ratios (1:1,400 and greater) and at all ratios tested of *H. lanata*, complete lysis of the entire erythrocyte sheet was seen (Fig. 3). Control erythrocyte preparations, without added pollen, were unchanged.

Hela cell monolayers incubated in the presence of pollen extracts showed loss of cells and staining with trypan blue of the remaining cells.

Biochemistry of haemolysin

Most of the haemolytic activity (assessed by titration in perspex plates as per p. 478) was found in the acetone-insoluble portion of the aqueous extract. Since the haemolytic activity passed an "Amicon" UMO5 ultrafilter the haemolytic molecules were evidently smaller in MW than about 500Da. On biogel P2 chrom-

atography of *Lolium perenne* and *Dactylis glomerata* extracts, each separated into three haemolytic fractions, which we entitled L1, L2 and L3 for the former species and D1, D2 and D3 for the latter (Fig. 4). L3 and D3 turned the laked haemoglobin greenish in colour.

L1, D1 and L3 were subjected to preparative layer chromatography on silica gel. L1 and D1 each split into seven bands (revealed by charring), of which the fastest-migrating band in each case contained the haemolysin. L3 was resolved into five bands, of which only the non-migrating portion was haemolytic. Amino acid analysis of the haemolytic portion of L1 (which we named L1,1) revealed alanine, aspartic acid, sarcosine, glutamic acid, valine, serine and isoleucine in molar ratio 6.48, 5.0, 2.1, 1.78, 0.74, 0.47, 0.05, as well as one unidentifiable peak. An artificial mixture of these seven amino acids was made up in barbitone buffer and tested for haemolytic capacity at the concentration calculated to be present in L1,1. Partial haemolysis was observed.

Weight and yield

Pollen grains were found to weigh between 10 ng and 100 ng, which agrees with earlier reports (7) varying from pollen to pollen and year to year (heavier in hot dry summers). Freeze-dried deionized water extracts of *Lolium perenne*, *Dactylis glomerata* and *Phleum pratense* yielded 0.27 g, 0.51 g and 0.46 g respectively per gram dry pollen. Of the 0.27 g *Lolium perenne* extract, 0.24 g was acetone-insoluble (haemolysin-containing). Dry *Lolium perenne* pollen yielded 1.48% L1 and 0.19% L1,1, as determined by weighing the freeze-dried materials.

Allergenicity testing of L1

In the RAST 24.0% of ^{125} I-tagged anti-IgE was bound in the presence of 50 μ l saline, using *Lolium perenne* pollen-coupled discs and pooled atopic serum. When 50 μ l of concentrated L1 was added instead the binding was unchanged. Prick tests in appropriate atopic volunteers with concentrated L1 solution produced no reaction at 15 min.

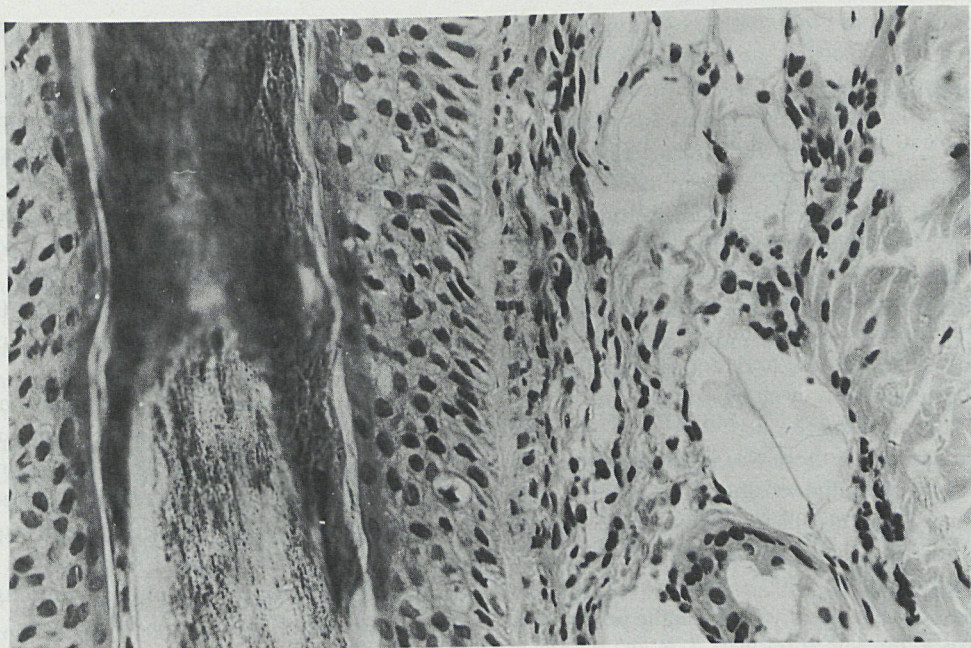


Fig. 5. Section of healthy human dermis 24 h after injection of 1–10 μ g of L1 toxin, showing polymorph infiltration of tissues surrounding hair shaft. Magnification approx. 90 $\times$.

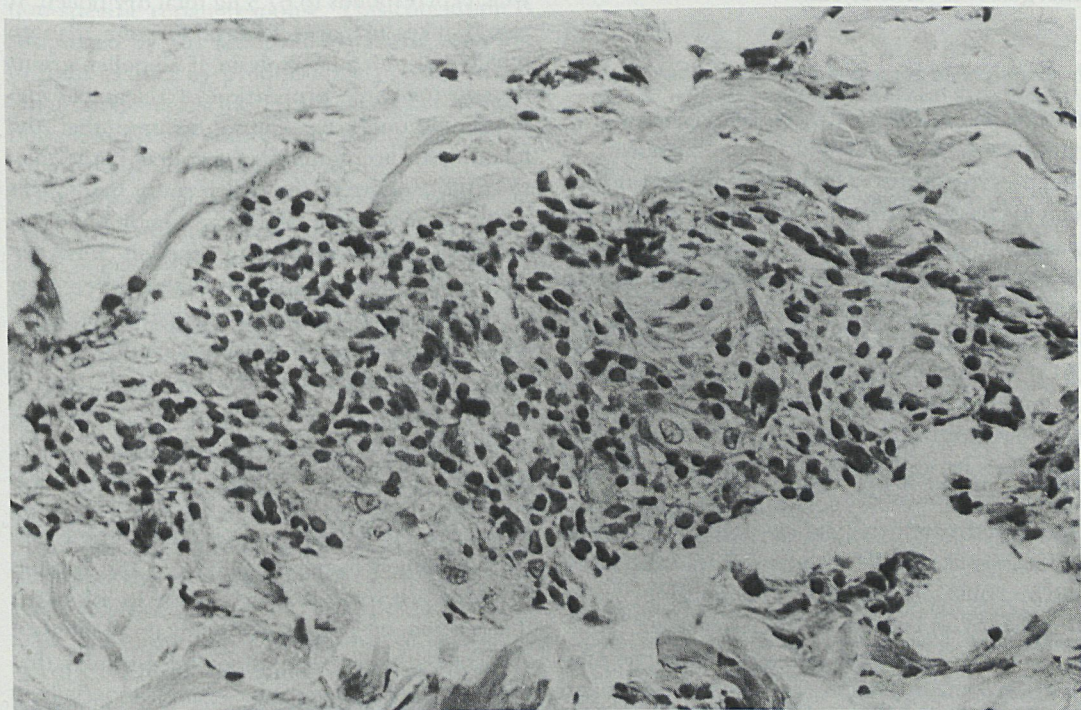


Fig. 6. Section of healthy human (D.L.J.F.) skin 2 weeks after injection of 150 μ g allergen-depleted *D. pteronyssinus* haemolysin, showing infiltration by lymphocytes and occasional macrophages. Magnification approx. 84 $\times$.

Toxicity in vivo of L1

Intradermal injections of L1 (0.1 µg) in sterile saline produced no naked-eye skin change in 13 volunteers. A dose of 1.0 µg produced a red patch, up to 3 mm dia., maximal at 6–24 h, in seven volunteers. At the higher dose of 10 µg, 11 reactions were seen in 15 volunteers. At this strength three of the volunteers stated that the patches were tender, and another had a soft non-reddened skin swelling some 2 cm in diameter surrounding the site.

Microscopically there was infiltration of polymorphs, with some eosinophils, around hair follicles, sweat glands, small nerves, erector pilae muscles and small blood vessels (Fig. 5). Control injections of saline produced no macroscopic change and only slight polymorph infiltration microscopically.

In this small series of volunteers, the presence or absence of clinical allergy was not correlated with the degree of skin reactivity to allergen-free haemolysin.

Experiments on housedust mite

Barbitone buffer extracts of each of two batches of *D. pteronyssinus* were haemolytic to sheep erythrocytes. The bulk of the haemolytic activity passed through an Amicon UMO5 ultrafilter and appeared in a single peak from the "Biogel P2" column. 3.0 g of dry mites yielded 7.5 mg of this haemolysin (i.e. 0.15% of dry weight). A RAST with *D. pteronyssinus* discs and D.L.J.F. serum at 1/4 (10% radioactivity bound) was unaffected by the addition of this material, and there was virtually no 15-min response to intradermal injection (150 µg). There was, however, a reaction at 6 h, with a tender red patch that finally disappeared after about 2 weeks. At 24 h there was infiltration by polymorphs and occasional eosinophils, as in the case of pollen lysin. At 2 weeks the polymorphs had been replaced by lymphocytes and occasional macrophages (Fig. 6). Pre-treatment with 60 mg prednisolone had no effect, macroscopic or microscopic, on this reaction.

Toxic dose of pollen in relation to natural exposure

On a hot June day in Manchester, the air contains about 300 pollen grains per cubic meter (0.3 grains per litre) throughout the daylight hours (Environmental Health and Control Department, City of Salford), most of which are of *Phleum pratense*. A non-allergic man breathing through his nose at a rate of 600 l/h will therefore transfer some 2880 pollen grains through that organ during 16 daylight hours. Because of the turbulent air flow (8) of the nose, most of the particles are deposited on the nasal and pharyngeal mucous membranes, but for the sake of conservatism let us say 50%. These will be removed by mucociliary action at a rate of 6–8 mm/min (9), allowing ample time for haemolysin release into the mucus. Assuming an average weight of 20 ng per pollen grain, the total daily exposure of this moderately active man is therefore 28.8 µg (total dry weight). The smallest dose of L1 lysin found to be inflammatory on intradermal injection was 1.0 µg, which corresponds to 67.5 µg total dry pollen. It would take just over 2 days for our man to inhale that much haemolysin, if all pollen grains contain the same proportion of releasable material, although we cannot assume that the haemolysin accumulates. Of course if the man were more active or the pollen count higher this would increase his intake correspondingly. Connell's calculated exposure of 6 pollen "hits" per minute (10) would quadruple the dose. Also, there are probably other toxic/inflammatory molecules that we have not included in this calculation (e.g. L2, L3 etc.).

DISCUSSION

25 batches of pollen, from eight different species, contained haemolysins, as did two batches of housedust mite. It is reasonable to infer that this is a general property of many or all inhalant allergens, although it cannot be assumed that the lysins are always active *in vivo*, especially in those pollens whose lysins were not apparent until after heating.

The chemistry of the lysins is still unknown. As the amino acids found within L1,1 are themselves partially haemolytic, it may be that this haemolysin is composed of amino acids. Although the extracts tested were sterile, some or all of the lysins may have been derived from pollen-borne micro-organisms since the dry pollens were frequently contaminated by bacteria and fungi.

The pollen fraction L1 and a corresponding fraction of housedust mite both produced inflammatory responses to intradermal injection, even though both fractions are devoid of detectable allergens. The type of response is non-specific, being similar to (but far greater than) that following the minor trauma of physiological saline injection. It is most unlikely that the skin response is an Arthus phenomenon, since precipitable antigens are invariably rather large ($MW \geq 5,000$ Da (11)), and molecules that large cannot have survived the purification process of L1. Presumably, the haemolysins (or some other component of these low MW fractions) are non-specifically cytotoxic and excite a non-specific repair response. This conclusion is supported by our failure to affect the response to mite extract by prednisolone pre-treatment. Small toxic molecules (such as alkaloids and polyphenols) are common in plants, so it is not surprising to find some in pollen.

Respiratory "allergic" diseases may not therefore be entirely immunologically mediated. This suggestion was independently made by Drake-Lee et al. (12), who elicited histamine release from nasal polyp tissue with allergen challenge when in some cases it could not be produced by anti-IgE challenge. Inhaled grass inflorescences produce a characteristic insidious bronchiectasis unlike other foreign body inhalations, whether or not the patient is allergic to grass (13), and bronchial biopsies in farmer's lung reveal focal areas of necrosis juxtaposed to inhaled pollen spores (14). Pollen also contains many enzymes (15) and at least one lectin (16). Exposure to allergenic particles causes bronchial hyper-reactivity in asthmatics (i.e. an increased sensitivity to histamine, methacholine, etc.) just like virus infections and poisonous gases. All of these find-

ings support a role for some direct cytotoxicity by the allergen-bearing particles. The aggregation of erythrocytes seen in Fig. 2 is presumably caused by the protein and lipid pollen haemagglutinins reported elsewhere (16).

The symptoms of Type I respiratory allergy may be interpreted as being protective, expelling toxic environmental particles from the mucous membranes (17). It may be that long-term suppression of hay fever and asthma by effective drugs is not entirely safe.

ACKNOWLEDGEMENTS

This work was funded by grants to D.L.J.F. from E. Merck Ltd., Alton, GU341JD, U.K. and to D.H.K. from NIH (AI-19476). Technical help was given at various times by Mrs P. Standart, Miss A. Davies, Miss E. Rigg and Mr E. Carter. Dr. P. Wilson did the RAST-inhibitions and the photomicrography. Dr. P. Brencley did some of the gel filtration. Dr. R. S. H. Pumphrey, Dr G. Taylor, Prof. R. R. A. Coombs, Dr. E. B. Bell and Prof. W. L. Ford gave helpful criticism and Mrs C. Bradburn coped with several drafts of the manuscript. This is publication No. 27 from the Medical Biology Institute, La Jolla, California.

REFERENCES

1. Coombs, R. R. A. & Gell, P. G. H.: Classification of allergic reactions responsible for clinical hypersensitivity and diseases. In Gell, P. G. H., Coombs, R. R. A. & Lachmann, P. J. (eds.): *Clinical aspects of immunology*, p. 768. Blackwell, Oxford, 1975.
2. Noon, L.: Prophylactic inoculation against hay fever. *Lancet* *i*, 1572-1573, 1911.
3. Dresser, D. W.: Assays for immunoglobulin-secreting cells. In Weir, D. M. (ed.): *Handbook of experimental immunology*, pp. 28.6-28.7. Blackwell, Oxford, 1978.
4. Oxoid manual, p. 94-95. Oxoid Ltd., Basingstoke, U.K., 1981.
5. Pepys, J.: Skin tests in diagnosis. In Gell, P. G. H., Coombs, R. R. A. & Lachmann, P. J. (eds.): *Clinical aspects of immunology*, pp. 55-58. Blackwell, Oxford, 1975.
6. Blackley, C. H.: Hay fever, p. 116 (2nd ed.). Baillière Tindall & Cox, London, 1880.
7. Morrow-Brown, H. & Irving, K. R.: The size and weight of common allergenic pollens. *Acta Allergol.* *28*, 132-137, 1973.
8. Proetz, A. W.: Air currents in the upper respiratory tract and their clinical importance. *Ann. Otol. Rhinol. Laryngol.* *60*, 439-467, 1951.
9. Quinlan, M. F., Salzman, S. D., Swift, D. L., Wagner, H. N. & Proctor, D. F.: Measurement of mucociliary function in man. *Am. Rev. Respir. Dis.* *99*, 13-23, 1969.

10. Connell, J. T.: Allergic rhinitis, human experimental model. *N.Y. State J. Med.* 70, 1751-1760, 1970.
11. Cunningham, A. J.: Understanding immunology, p. 9. Academic Press, New York, 1978.
12. Drake-Lee, A. B., McLaughlan, P. & Barker, T. H. W.: Histamine, asthma, and nasal polyps. *Lancet* *ii*, 213, 1982.
13. Dudgeon, D. L., Parker, F. B., Fritelli, G. & Rabuzzi, D. D.: Bronchiectasis in pediatric patients resulting from aspirated grass inflorescences. *Arch. Surg.* 115, 979-983, 1980.
14. Heino, M., Mönkäre, S., Haahtela, T. & Laitinen, L. A.: An electron-microscopic study of the airways in patients with farmer's lung. *Eur. J. Respir. Dis.* 63, 52-61, 1982.
15. Bousquet, J., Guerin, B., Hewitt, B. & Michel, F. B.: Analysis of commercial pollen extracts by enzyme determination I. Comparison of RAST-inhibition assay and enzyme titration for orchard grass, rye grass and short ragweed pollen extracts. *Ann. Allergy* 45, 310-315, 1980.
16. Freed, D. L. J.: Lectins, allergens and mucus. In Bøgg-Hansen, T. C. (ed.): *Lectins: Biology, biochemistry, clinical biochemistry*, pp. 33-43. Berlin, Walter de Gruyter, 1982.
17. Freed, D. L. J.: The immunology of allergy. In Rees, A. R. & Purcell, H. (eds.): *Disease and the environment*, pp. 65-78. Wiley, Chichester, 1982.

Address:

Dr. D. L. J. Freed

Dept. of Bacteriology & Virology

University of Manchester

Medical School

Oxford Road

Manchester M13 9PT

U.K.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.