

Allergy to guinea pigs: I Allergenic activities of extracts derived from the pelt, saliva, urine and other sources

A. F. WALLS, A. J. NEWMAN TAYLOR* and JOAN L. LONGBOTTOM

*Department of Allergy and Clinical Immunology, and *Department of Occupational Medicine, Cardiothoracic Institute, Brompton Hospital, London SW3, U.K.*

(Received in original form 24 July 1984 and in revised form 14 November 1984; accepted for publication 10 December 1984)

Summary

Guinea pig-sensitive patients with asthma and rhinitis were skin test positive to extracts of several materials derived from guinea pigs. A radioallergosorbent test (RAST) was developed to measure serum IgE specific for the dander, urine, saliva and also for dust from the air-vent filters of a room housing guinea pigs. A strong correlation was found between positive skin test reactions, and raised serum IgE to these extracts. Furthermore, the relative allergenic potency of extracts was similar when determined by skin-prick testing and by inhibition of the RAST to guinea pig dust.

Non-guinea pig-derived extracts such as the hay, sawdust and diet had negligible activity in skin testing and RAST inhibition; and preparations of *Dermatophagoides pteronyssinus*, house dust and rat dust did not inhibit the RAST for guinea pig room dust.

The guinea pig dust, dander, fur, urine and saliva were the more potent extracts; while whole pelt, faeces and serum were considerably less active. Extracts from different sexes were not appreciably different in potency.

The results of skin testing, RAST and RAST inhibition suggest cross-allergenicity between the various extracts. Although material shed from the pelt may have been derived from saliva, or even urine, allergenic activities of urinary and salivary preparations were found to be less than those of the dander, fur or dust. This suggests that allergens have become concentrated on the pelt.

Introduction

The capacity of the guinea pig to provoke asthmatic reactions in a sensitized subject was recognized by Salter as early as 1868 [1]. He ascribed the effect to the smell emanating from the animals.

Correspondence: Dr A. F. Walls, Department of Biology, York University, Heslington, York, YO1 5DD, or Joan L. Longbottom, Department of Allergy and Clinical Immunology, Cardiothoracic Institute, Brompton Hospital, London SW3, U.K.

Since that time, there have been many reports in which exposure to guinea pigs has been implicated as a cause of allergic disease. These have used a variety of guinea pig-derived materials in skin testing, bronchial provocation and serological investigations, to diagnose the condition.

Extracts of hair clipped from guinea pigs have been used for skin tests [2–18]. To investigate the properties of guinea pig allergens, whole pelt has been extracted [17].

The allergenic activity of serum is also recognized. Serum preparations have frequently been used for skin testing [17–22] and for serological investigation [23].

Following the discovery of the allergenic activity of proteins excreted in the urine of rats and mice [24, 25] guinea pig urine extracts have been used for skin testing, in the radioallergosorbent test (RAST) [17, 21, 22, 26] and in bronchial provocation testing [27]. Moreover, RAST inhibition of urine has been used to estimate levels of guinea pig allergens in dust samples [28].

Saliva has received little attention as a source of guinea pig allergens although contact urticaria was provoked in a guinea pig-sensitive patient after a guinea pig was allowed to lick her skin [13]. Even reactions with the faeces have been noted [6].

Many source materials have been used diagnostically. Despite the diversity of the extracts employed, there have been few attempts to compare allergenic activities, or to determine which are most suitable for diagnostic purposes. Skin tests have been performed with more than one source material, such as whole pelt and serum [18] urine and serum [22] and urine, fur and serum [17, 21]; but these studies have not provided a basis for evaluation of different materials. Small numbers of guinea pig-sensitive patients were investigated; and in most cases the extracts used were not directly comparable in their concentration, their method of preparation, or animals from which they were derived.

The aim of this study has been to investigate and compare extracts of materials collected from or associated with guinea pigs. A RAST for IgE specific to various extracts was developed, and allergenic activities were measured by skin-prick testing and by RAST inhibition of guinea pig room dust.

Materials and methods

Source materials

All extracts were derived from at least two Dunkin Hartley guinea pigs (3 star, Redfern, Frant, Sussex), aged 10–16 weeks (post-pubertal). Mean weights for male animals were 650 g (range: 590–800 g) and for females, 560 g (range: 535–580 g).

Guinea pigs were killed by cervical dislocation, and either fur was clipped from the flanks, using electric clippers, or whole pelt (the entire skin and hair from which extraneous fat had been excised) was collected.

Guinea pigs tend to urinate and defaecate in just one corner of a cage, making it possible to collect material shed naturally from the animal's coat. Guinea pigs were housed on wire mesh, in rectangular cages below which was placed a sloped stainless steel tray with drainage at one corner. A layer of dry skin scales, fur and small crumbs of pelleted diet accumulated on the tray. This 'dander' material, excluding that deposited near to the corner contaminated with urine or faeces, was collected every 24 hr.

Rat metabolic cages (Tecniplast, Varese, Italy), were adapted to house guinea pigs, and used to collect urine. It was necessary to dispense with the corridors leading to diet

and water; to convert one corridor into a food hopper and to insert a water bottle through the roof of the cage.

Salivation was induced with pilocarpine nitrate (McFarlan Smith, Edinburgh) (10 mg injected subcutaneously) after anaesthesia with ketamine hydrochloride (Ketalar, Park-Davis, Pontypool) (15 mg injected intramuscularly). Between 3 and 5 ml saliva was withdrawn from the mouth on each of several occasions, at least 1 week apart. Animals were not allowed food for 1 day before collection in order to minimize contamination with diet and to reduce the risk of regurgitation. When regurgitation did occur, the saliva was discarded. Analytical isoelectric focusing (LKB PAG plates pH range 3.5–9.5, LKB, Bromma, Sweden) of samples collected at different times, did not demonstrate qualitative differences in the components present.

Pilocarpine-stimulated guinea pig faeces were collected at the same time as the saliva. Faecal pellets extruded within the first 5–10 min of the treatment were collected directly into a glass container. Serum was obtained from blood withdrawn from the heart under ether-induced anaesthesia.

Dust was collected from the filter in the air vent of a large guinea pig breeding room. The guinea pigs were supported on wire mesh floors, placed over trays for collection of urine and faeces. These were rinsed out regularly. No other animal species were housed on that site. Fresh unused sawdust, hay and diet (SDS Diets, Witham, Essex) were collected (though sawdust was never used in the room which the dust sample was obtained). All material was stored at -20°C , until extracts were prepared.

Extraction

Extracts were prepared essentially as previously described [29]. Solid source materials were freeze-dried before incubation in diethyl ether (1:10, W:V) for 24 hr, dried at room temperature, and extracted (1:10, W:V) in Coca's solution pH 7.2 (0.5% sodium chloride; 0.275% sodium hydrogen carbonate; 0.4% phenol) for 3 days at room temperature.

Extracts in Coca's solution, and urine, saliva and serum were passed through $0.45\ \mu$ membrane filters (Oxoid, London), dialysed for 72 hr at 4°C in several changes of 0.05 M ammonium hydrogen carbonate (pH 7.8), and freeze-dried. Storage was at 4°C in the dry form.

Patients and sera

The patients all had rhinitis and the majority asthma provoked by exposure to guinea pigs. They were skin-test positive to guinea pig-derived extracts, and had been exposed to guinea pigs either as laboratory animals or as pets. Most patients had had contact with other small mammals, especially with the rat, mouse and rabbit; and sensitivity as determined by clinical history, skin-prick testing and RAST, was commonly to more than one of these species.

Venous blood was allowed to clot overnight, before centrifugation and aspiration of serum. Storage was at -20°C or at 4°C with 0.05% (W:V) sodium azide.

Skin-prick testing

Extracts were dissolved in Coca's glycerol (1:1), heated at 56°C for 30 min to denature viruses, and filtered ($0.45\ \mu$) (Millipore, Bedford, Mass., U.S.A.).

Skin-prick testing was performed on the volar aspect of the forearm, and sites were randomized. The various extracts were tested at 0.01 mg/ml, and the concentration was

increased to 1 mg/ml only if a positive reaction was not elicited. A weal diameter of 3 mm or greater after 20 min was considered positive. Weal area was estimated from the mean diameter.

RAST

A RAST was developed to measure serum IgE specific for guinea pig dust, dander, urine and saliva. Freeze-dried allergen extracts were covalently coupled to cyanogen bromide activated Sepharose 4B (Pharmacia, Uppsala, Sweden). The activated Sepharose was allowed to swell for 15 min in 10^{-3} M hydrochloric acid, before washing in a scintered glass funnel with the same solution. Moist swollen gel was transferred to a container with 2 mg/ml extract in 0.1 M sodium hydrogen carbonate/0.5 M sodium chloride buffer (pH 8.5) (10 mg extract/g Sepharose, by dry weight) and rotated overnight at 4°C.

Excess extract was removed by washing the allergosorbent with the bicarbonate coupling buffer; and any sites of cyanogen bromide which had not been coupled to the extract were blocked by incubation in 1 M ethanolamine in coupling buffer (pH 8) for 2 hr at room temperature. The gel was washed with coupling buffer (pH 8.5), acetate buffer (pH 4) (0.1 M sodium acetate, 1 M sodium chloride) and then borate buffer (pH 8) (0.1 M boric acid, 1 M sodium chloride) and the cycle repeated two more times (approximately 30 ml of each buffer per g dry Sepharose on each cycle).

The allergosorbent was stored in a stock concentration of 25% in phosphate buffered saline (PBS) containing 0.02% sodium azide and 0.3% human serum albumin.

The conditions for RAST were determined by titrating the allergosorbent, patients' sera and ^{125} I-labelled anti-IgE (Pharmacia) [29, 30].

With all of the extracts coupled to Sepharose, 100 μ l of 5% allergosorbent were incubated with 50 μ l of patients' sera in duplicate tubes (LP4, Luckham, Burgess Hill, Sussex), and shaken at room temperature for 16 hr. Three ml normal saline, containing Tween 20 (5%), were added to each tube for 10 min, and centrifuged at 500 g for 8 min. This washing procedure was repeated three times.

Fifty μ l ^{125} I-anti-IgE (Pharmacia) were added to each tube, incubated with shaking for 16 hr, washed three times as before, and tubes were counted for 2 min in a gamma counter.

RAST inhibition

One hundred μ l aliquots of pooled patient or control sera were added to 100 μ l of extract in different concentrations in PBS, and incubated on a shaker at room temperature. After 3 hr, the contents of each tube were divided equally between two tubes, 100 μ l of 5% allergosorbent were added to each, and the RAST procedure performed as above.

The percentage inhibition in the system was estimated as:

$$\frac{(A - C) - (a - c)}{A - C} \times 100$$

where *A* and *C* were the counts obtained for allergic and control sera respectively, without the addition of inhibiting extract; and *a* and *c* were the counts for the allergic and control sera with inhibiting extract added.

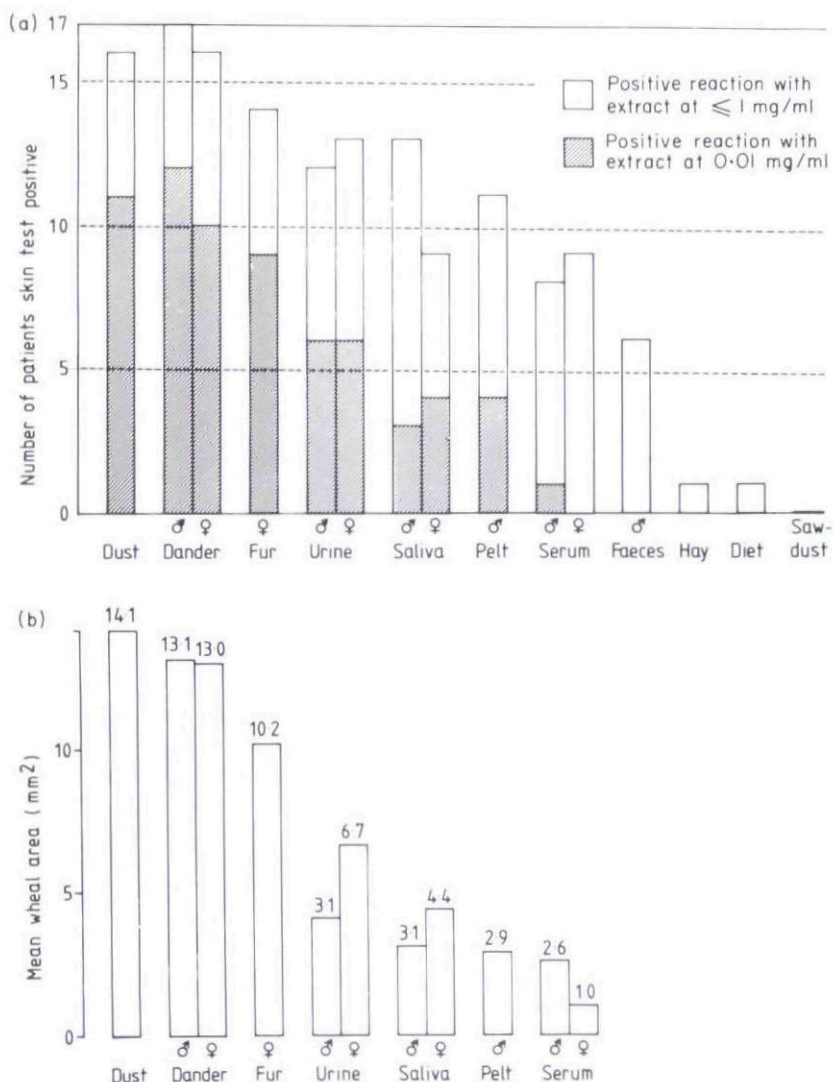


Fig. 1. Skin-prick test reactions elicited by a range of guinea pig allergen extracts: (a) total numbers of positive reactions in seventeen guinea pig-sensitive patients tested at extract concentration of 0.01 mg/ml and at 1 mg/ml; (b) mean wheal area in fourteen patients, with each extract at 0.01 mg/ml.

Results

Skin Tests

Seventeen patients allergic to guinea pigs, all with rhinitis and sixteen with asthma had immediate skin-prick test reactions to extracts of female guinea pig dander (Fig. 1a). All but one reacted to male guinea pig dander and to extracts of dust from the room housing guinea pigs tested at concentrations of 1 mg/ml or less. Fur provoked reactions in fourteen patients, urine extracts in twelve and thirteen, saliva in twelve and nine, whole pelt in eleven, serum in eight and nine and faeces in six. One of the patients responded to hay and diet. No reactions were elicited by sawdust.

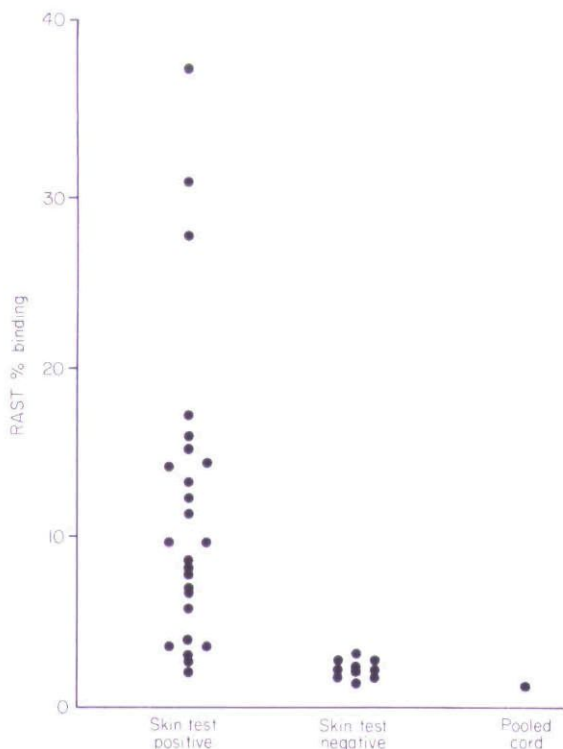


Fig. 2. RAST for IgE specific for guinea pig room dust, with sera from twenty-five patients who were skin test positive to guinea pig extracts, and from ten skin test negative control subjects; and with pooled cord serum.

The mean weal areas for fourteen of these patients (thirteen with rhinitis and asthma and one with rhinitis) who reacted to extracts tested at 0.01 mg/ml, are also shown in Fig. 1b.

Skin-prick tests in fifteen asymptomatic control subjects with the full range of guinea pig source materials, at a concentration of 1 mg/ml, provoked no positive reactions. Five of these subjects were atopic, and five regularly exposed to guinea pigs.

RAST

A highly significant relationship ($P < 0.001$, Mann-Whitney U test [31]) was found between positive skin test reactions to guinea pig-derived materials and high levels of serum IgE specific for these extracts (Fig. 2).

IgE RAST values are shown for eighteen asthmatic patients who were skin-prick test positive to guinea pig extracts (Table 1).

A significant correlation (Spearman's rank correlation coefficient) was found ($P < 0.01$) between relative RAST scores, with all possible pairings of these four different guinea pig source materials.

RAST inhibition

Inhibition of the RAST for IgE specific for dust from a guinea pig room was carried out using a pool of serum from twelve guinea pig allergic patients, and another from twelve

Table 1. % binding in RAST for IgE specific for guinea pig dust, dander, saliva and urine

Guinea pig-allergic patients	Mean % binding			
	dust	dander	saliva	urine
PV	29.9	30.8	40.2	31.8
RF	20.2	26.9	25.4	22.8
PE	16.1	16.4	31.6	17.1
AB	15.0	17.5	14.1	16.4
GS	13.9	17.9	18.2	17.1
PK	11.8	15.3	13.6	10.6
CS	10.0	23.0	20.5	16.0
JS	9.3	12.3	7.6	10.3
JP	9.2	10.4	10.6	10.0
HF	8.8	18.0	6.3	6.5
CA	8.8	14.0	1.0	3.0
RC	8.2	10.9	15.0	9.7
RB	6.5	18.4	11.0	9.1
IB	6.1	13.3	8.3	11.4
TB	4.4	7.5	2.0	4.0
DC	3.2	10.5	1.2	6.3
RA	1.9	1.7	1.1	1.6
GA	1.8	9.0	3.3	3.9
10 control sera:				
mean	1.0	1.2	0.7	1.3
Upper 95% confidence limits	1.8	1.7	1.1	1.9
Pooled cord sera	0.8	1.2	0.4	1.5

control subjects. Results were very similar when the assay was carried out on two separate occasions. Mean % inhibition (combining the results of both assays) was plotted for each extract (Fig. 3). Pre-incubation of the control sera with these extracts did not inhibit the RAST results. Mean RAST binding for the control serum pool was 0.8% and for the allergic pool, 20.7%.

The inhibitory effects of dust and the male dander extract tested were similar. The female fur preparation and extracts of male guinea pig urine, saliva, whole pelt, serum and faeces were less active on a dry weight basis.

Pre-incubation of the allergic serum pool with extracts of hay, guinea pig diet and sawdust had negligible effect; and no cross-allergenicity in this system was discovered between guinea pig room dust and rat room dust, house dust or the house dust mite, *Dermatophagoides pteronyssinus*.

Discussion

Close relationships were found between positive skin-prick test reactions to guinea pig extracts, the presence of specific IgE to these extracts and asthma provoked by guinea

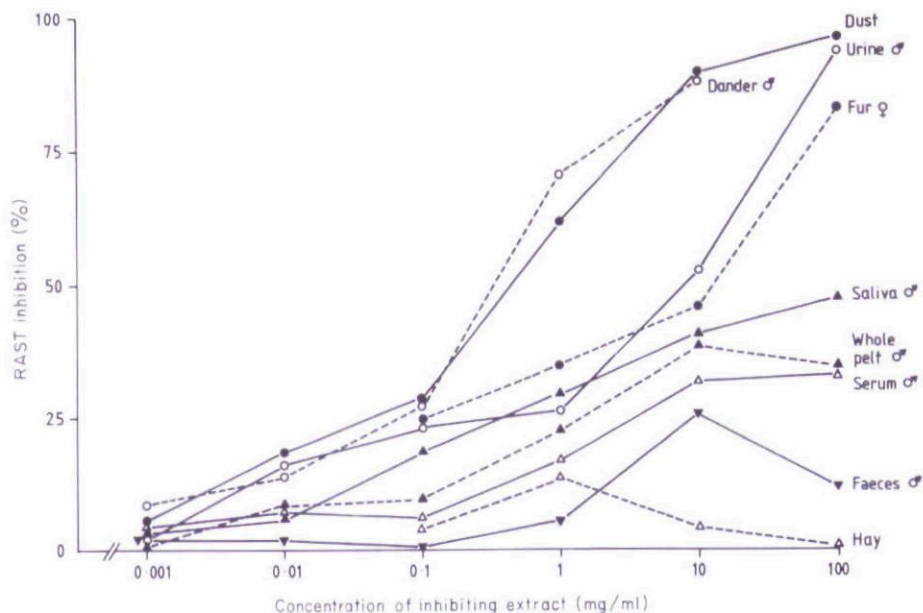


Fig. 3. Inhibition of the RAST for IgE specific for guinea pig dust, when extracts of various materials derived from or associated with guinea pigs, were added.

pigs. Previous investigations have failed to find a consistent relationship between allergic symptoms and the results of skin tests and RAST to guinea pig extracts [11, 12, 15, 16]. Possible reasons for discrepancies in the findings are differences in patient selection, and the performance of the RAST. Asthma, but not rhinitis or conjunctivitis, seems to be associated with positive skin test reactions to laboratory animal extracts [14, 21, 22] and all but one of our patients had asthma.

The dust which was collected may be considered representative of inhalant material, hence inhibition of the guinea pig dust RAST should provide a measure of the allergenic activity of other relevant extracts; and the findings of RAST inhibition were consistent with the skin test data.

The selection of an extract which is the most appropriate for use in diagnosis by RAST, skin-prick or other challenge tests, is complicated by the demonstration of allergenic activity for materials from such a wide range of sources. Extracts of materials associated with the husbandry of guinea pigs, however, had little skin test activity and caused negligible inhibition of the guinea pig dust RAST. Only one of the patients reacted to extracts of diet and hay at the maximum concentration, while none reacted with sawdust. Moreover, preparations of *D. pteronyssinus*, house dust and even dust from a rat room had no effect on the guinea pig dust RAST inhibition, although the use of pooled sera may have masked significant inhibition for individual patients. Nevertheless, for the group of patients investigated, the principal inhalant allergens in the guinea pig room, were derived from guinea pigs.

On a dry weight basis, allergenic activity was highest for extracts derived from the pelt, urine and saliva; and the sex of the source animal had negligible effect on allergenicity. The preparations of serum and faeces were of relatively low potency.

These findings are in accord with other more limited observations. Ohman *et al.* [18], reported that all of nine guinea pig-sensitive patients studied reacted on skin testing to a pelt extract, but only five reacted to serum; and in an epidemiological investigation, Beeson *et al.* [17] found that five subjects reacted to extracts of guinea pig urine, four to fur, and four to serum.

Guinea pig dust, dander, urinary and salivary extracts all proved most effective in both skin testing, and RAST. The observation of multiple sensitivity to these guinea pig allergen extracts, the extent of RAST inhibition by the different preparations, and the finding of close correlation between RAST results suggest that the major allergens of these more potent source materials are shared. This conclusion has been supported by immunoelectrophoretic analysis [29, 33].

The allergens of the guinea pig appeared to be most concentrated on the outer surface of the pelt, since on a dry weight basis, the allergenic activity of the fur extract was greater than that of the whole pelt; while the dander preparation proved even more potent. This suggests that the allergens of the guinea pig coat may have resulted, to a large extent, from contamination. The most likely contaminants of guinea pig fur will be saliva and urine, and there is probably an accretion of allergens from these sources on the outer layer. Salivary proteins in particular must be deposited in great quantity on that material of the pelt which is removed during grooming, or which is disturbed when the animals are handled.

If guinea pig allergens are derived predominantly from saliva or urine, however, it seems paradoxical that extracts of these materials should prove significantly less potent in skin tests and RAST inhibition than the extracts of dust, dander and fur. The degree of cross-allergenicity between those guinea pig extracts, and the antigenic similarities observed using QIE procedures [29, 33] make it improbable that the greater allergenic activity of dust, dander and fur was due to the cumulative effect of different potent allergens from various sources. Moreover, major guinea pig allergens identified by crossed radioimmunoelectrophoresis [29] have been detected in higher levels in the extracts of dander and fur, than in salivary or urinary extracts [32].

The tendency for fur and dust to be of higher allergenic potency than other source materials was less evident in a comparable study of rat allergen extracts [34]. It has been reported recently, however, that saliva and urine extracts derived from cats or dogs, can be of lower potency in RAST inhibition, than certain commercial dander preparations from these animals, when these are compared on a dry weight basis [35].

The results of quantitative immunoelectrophoretic studies show dust and dander extracts to be deficient in certain antigens present in saliva [32]. Similar findings with rat allergen extracts [34, 36], suggest that this could be a reflection of differential stability. The constituents of saliva which were of low allergenic activity may have become degraded, while the major allergenic components, being of greater stability, could thus have become more concentrated on the pelt.

The immunochemical nature of guinea pig allergen extracts is being studied further [33], with a view to determining more precisely the extent of antigenic cross-reactivity which these investigations appear to indicate. This study has confirmed that allergens from the guinea pig are distributed in many source materials and are most concentrated in dander and dust. Nevertheless, the saliva and possibly urine, may represent the origin of these allergens. If this is the case, then one extract of either fur, saliva or urine should prove adequate for diagnostic use if employed in a sufficiently high concentration.

Acknowledgments

We gratefully acknowledge clinical help from Miss Rosemarie Hawkins, Dr M. Dally, Dr R.E.C. Altounyan, Dr A.J.M. Slovak, Dr C.A.C. Pickering and Professor A.B. Kay. We thank also Dr M.W. Elves and Dr D. Turner for their comments on this work, Miss Alex Tee for typing the manuscript, Mrs Rosemary Kay for artwork and the Photographic Service of the Royal Marsden Hospital.

Funding was provided by the Science and Engineering Research Council and Glaxo Group Research plc.

References

- 1 Salter JH. On asthma: its pathology and treatment. 2nd ed. London: John Churchill and Sons, 1868.
- 2 Markley AJ. Anaphylactoid dermatitis due to direct contact with animal epidermal structures. *Arch Dermatol Syphilol* 1920; 2:722.
- 3 Walker IC. Frequent causes and the treatment of perennial hay fever. *JAMA* 1920; 75:782.
- 4 Rackeman FM. Asthma. *Med Clin North Am* 1923; 7:765.
- 5 Feinberg SM. Allergy in practice. Chicago: Year Book Publishers, 1946.
- 6 Braun W. Allergie gegen Meerschweinchen. *Allerg Asthma (Leipz)* 1960; 6:176.
- 7 Böhm Von W, Braun W. Berufsbedingte sensibilisierungen der atemwege durch haare von labortieren. *Arbeitsmed Sozialmed Arbeitshyg* 1972; 7:94.
- 8 Kobayshi S, Toyoda T, Nakazawa T. Bronchial asthma caused by hair and dander from guinea pigs and rabbits. *Jpn J Allergol* 1972; 21:244.
- 9 Ishiyama H, Kato K, Furuya K, Shida T. Animal dander allergy found in a laboratory of a cosmetic company. *Jpn J Allergol* 1974; 24:7.
- 10 Bruchhausen Von D, Bruchhausen M. Beobach tungen über Meerschweinchen- und Goldhamster-haar-allergien. *Z Allgemeinmed* 1975; 51:1600.
- 11 Koers WJ, Young E, Berrens L. Animal dander allergy. *Clin Allergy* 1977; 7: 245.
- 12 Bruynzeel PLB, Berrens L. IgE and IgG₄ antibodies in specific human allergies. *Int Arch Allergy Appl Immunol* 1979; 58:344.
- 13 Rudzki E, Rebandel P, Rogozinski T. Contact urticaria from rat tail, guinea pig, streptomycin and vinyl pyridine. *Contact Dermatitis* 1981; 7:186.
- 14 Slovak AJM, Hill RN. Laboratory animal allergy: a clinical survey of an exposed population. *Br J Ind Med* 1981; 38:38.
- 15 Van Toorenenbergen AW, Aalberse RC. Allergen-specific IgE and IgG₄ antibody levels in sera and the capacity of these sera to sensitise basophil leucocytes *in vitro*. *Clin Allergy* 1982; 12:451.
- 16 Staud Von RD, Rudolph, R, Baumgarten C, Kunkel G, Sladek M. Immunoglobulin verläufe von gesamt-und spezifischen IgE bei tierhaar-allergikern nach tierhaarkarenz. *Allergol* 1982; 2:75.
- 17 Beeson MF, Dewdney JM, Edwards RG, Lee D, Orr RG. Prevalence and diagnosis of laboratory animal allergy. *Clin Allergy* 1983; 13:433.
- 18 Ohman JL, Lowell FC, Bloch KJ. Allergens of mammalian origin. II. Characterization of allergens extracted from rat, mouse, guinea pig and rabbit pelts. *J Allergy Clin Immunol* 1975; 55:16.
- 19 Simon FA. Human allergy to mammalian sera. *J Exp Med* 1942; 75:315.
- 20 Arbesman CE, Beede RB, Rose NR. Sensitivity to animals: A case report with immunologic studies. I. Clinical aspects. *J Allergy* 1958; 29:129.
- 21 Newman Taylor AJ, Myers JR, Longbottom JL, Spackman D, Slovak AJM. Immunological differences between asthma and other allergic reactions in laboratory animal workers. *Thorax* 1981; 36:229. Abst.
- 22 Cockcroft A, Edwards J, McCarthy P, Andersson N. Allergy in laboratory animal workers. *Lancet* 1981; i:827.
- 23 Beede RB, Rose NR, Arbesman CE. Sensitivity to animals: A case report with immunologic studies. II. Serologic aspects. *J Allergy* 1958; 29:139.
- 24 Frankland AW. Rat asthma in laboratory workers. *Excerpta medica* 1974; 123.
- 25 Newman Taylor AJ, Longbottom JL, Pepys J. Respiratory allergy to urine proteins of rats and mice. *Lancet* 1977; ii:847.
- 26 Edwards RG, Lee D, Beeson MF, Dewdney JM, Spackman DA. The development and validation of radioallergosorbent tests for the detection of specific human IgE antibody directed against laboratory animal urinary proteins. *Int Arch Allergy Appl Immunol* 1983a; 71:53.

- 27 Nagy L, Lee TH, Kay AB. Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. *N Engl J Med* 1982; 306:497.
- 28 Edwards RG, Lee D, Beeson MF, Dewdney JM. Laboratory animal allergy: the measurement of airborne urinary allergens and the effects of different environmental conditions. *Lab Anim* 1983b; 17:235.
- 29 Walls AF, Longbottom JL. Allergy to rats: a comparison of allergenic potencies for a range of source materials by skin prick testing, RAST and RAST inhibition. *J Allergy Clin Immunol*, (In press).
- 30 Gad El Rab MO, Kay AB. Widespread IgE-mediated hypersensitivity in the Sudan to the 'green nimitti' midge *Cladotanytarsus lewisi* (Diptera: Chironomidae). I. Diagnosed by radioallergosorbent test (RAST). *J Allergy Clin Immunol* 1980; 66:190.
- 31 Sigel S. Nonparametric statistics for the behavioural sciences. Tokyo: McGraw-Hill, Kogakusha, 1956.
- 32 Walls AF. Allergy to rats and guinea pigs: An immunochemical study of the sources and nature of allergens. PhD Thesis, University of London 1984.
- 33 Walls AF, *et al* Allergy to guinea pigs. II. Identification of specific allergens in guinea pig room dust by crossed radio-immunoelectrophoresis and investigation of their possible origin (unpublished observations).
- 34 Walls AF, Longbottom JL. Quantitative immunoelectrophoretic analysis of rat allergen extracts. I. Antigenic characterisation of fur, urine, saliva and other rat-derived materials. *Allergy* 1983a; 38:419.
- 35 Viander M, Valovirta E, Vanto T, Koiuikko A. Cross-reactivity of cat and dog allergen extracts. RAST inhibition studies with special reference to the allergenic activity in saliva and urine. *Int Arch Allergy Appl Immunol* 1983; 71:252.
- 36 Walls AF, Longbottom JL. Quantitative immunoelectrophoretic analysis of rat allergen extracts. II. Fur, urine and saliva studied by crossed radioimmunoelectrophoresis. *Allergy* 1983b; 38:501.

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.