

Original articles

Comparison of rat fur, urine, saliva, and other rat allergen extracts by skin testing, RAST, and RAST inhibition

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Extracts of a wide range of materials associated with exposure to rats were prepared and their relative allergenic activities were measured by skin-prick testing of rat-sensitive patients, RAST for serum IgE, and RAST inhibition of dust collected from a rat room. Most potent on a dry weight basis were preparations of fur, urine, epithelia, and saliva (all irrespective of the sex of the rat) and of the dust. Extracts of shaved pelt, whole pelt, feces, and serum proved less effective, whereas those of sawdust or diet had negligible activity. The presence of similar allergens in the more potent extracts was suggested by multiple skin sensitivity to different source materials, by close correlation between RAST results, and by the extent of RAST inhibition for individual extracts. The allergenicity of fur and epithelia probably results largely from contamination with saliva and urine. (J ALLERGY CLIN IMMUNOL 75:242-51, 1985.)

Allergy to laboratory animals represents a serious occupational health problem. Estimates of those in employment who suffer from symptoms of hypersensitivity to these animals range from 11.3% to 30%.¹⁻¹⁰ The rat is one of the animals most commonly used and is responsible for symptoms in a large proportion of those with disease.

There has been a widespread assumption that allergy to mammals results from sensitization to airborne skin scales. Hence several investigations of allergy to rats have used pelt-derived materials for purposes of skin-test diagnosis^{6, 10-14} and immunotherapy.^{11, 13, 14} Some physicochemical properties of allergens extracted from the whole rat pelt have been described by Ohman et al.¹⁵ who reported that all of a group of 11 rat-sensitive patients were skin test

Abbreviations used

APC: Allergen particle complex
S-D/G: Sprague-Dawley/Glaxo rat strain

positive to whole pelt, and some were skin test positive to serum as well.

Commercial skin-test preparations are mostly extracts of fur or epithelia. Recent work, however, has highlighted the possible importance of rat urine as a source of allergenic material. In a previous study of five rat-allergic patients, an extract of urine proved more effective than those of hair or serum in the elicitation of positive skin and bronchial provocation tests,¹⁶ and two major rat urinary allergens have been characterized.^{17, 18} Urine has also been implicated as an important source of mouse allergens,¹⁶⁻²⁰ and epidemiologic surveys of laboratory animal allergy have indicated a close correlation between symptoms and skin test positivity to urines.^{7, 8, 10, 21}

In the measurement of rat-derived material in dust samples, assays have been developed for both skin^{22, 23} and urinary²³⁻²⁵ allergens. Other potential sources of rat allergens have been largely ignored. Nevertheless, there is evidence for the salivary origin

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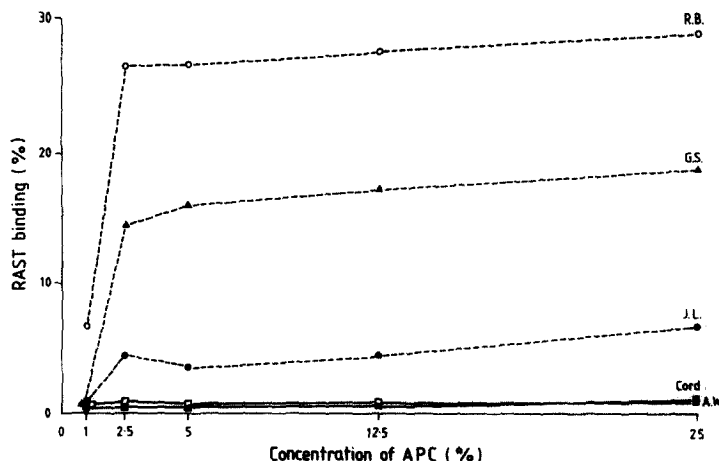


FIG. 1. Graph of percent binding in RAST versus concentrations of rat dust APC (100 μ l) for sera (50 μ l neat) of three rat-sensitive patients (R. B., G. S., J. L.), a control subject (A. W.), and pooled cord serum; 50 μ l ^{125} I-labeled anti-IgE (37,000 counts per 2 min) was added.

of allergens from certain other mammalian species, such as the cat,²⁶⁻²⁹ dog,^{26, 29} rabbit,²⁶ and guinea pig.³⁰ Moreover, it has been reported that systemic anaphylactic shock can result from a rat bite,³¹ and urticarial reactions have been noted around bites.¹

The quantity of fecal material produced by rats has been considered in the context of allergy to this species³² but has not otherwise attracted attention. Similarly, the role played by rat diet and bedding materials in the provocation of allergic reactions has not been investigated, although it has been suggested that sensitivity to animal- or plant-derived foodstuffs may be relatively common.³³

The aim of this study was to prepare a wide range of extracts relevant to exposure to rats and to develop a RAST for IgE specific for these extracts. The material collected was derived from rats of the same age and strain, and extracts were prepared under similar conditions. Potencies were compared by skin-prick testing, RAST, and RAST inhibition. The results of quantitative immunoelectrophoretic analysis of these rat-derived extracts³⁴ and of crossed radioimmuno-electrophoresis with rat fur, urine, and saliva³⁵ have been reported separately.

MATERIAL AND METHODS

Collection of material

Ten- to 14-wk-old S-D/G rats (Glaxo Group Research, Harefield, U. K.) were the source of all animal extracts. Weights of male rats ranged between 280 and 415 gm (mean 340 gm) and of female rats between 240 to 275 gm (mean 260 gm). Each rat-derived source material was collected and pooled from at least four rats.

Along with *whole rat pelt*, fur clipped off close to the skin with electric clippers, *epithelial scales* removed by

scraping with a razor blade, and *shaved pelt*, which was the skin that had been scraped, were collected, and 24-hour collections of rat urine were obtained by use of metabolic cages (Tecniplast, Varese, Italy) and pooled. A small volume was collected by bladder massage for comparison.

Saliva was withdrawn directly from the mouth through silicone rubber tubing after its stimulation by pilocarpine nitrate (4 mg injected subcutaneously) (McFarlan Smith Ltd., Edinburgh, U. K.) under ketamine hydrochloride anesthesia (25 mg injected intramuscularly) (Ketalar; Parke-Davis, Pontypool, U. K.). Despite the high dose of ketamine hydrochloride required for immobilization of rats,³⁶ this anesthetizing agent was used because it enhances the production of saliva. Administration of certain other commonly used anesthetics (e.g., pentobarbital sodium) actually inhibits salivation. Three to 4 ml of saliva was collected from each rat on several occasions, an interval of at least 10 days elapsing between each collection.

Extrusion of *feces* directly into a glass container was provoked by handling the rats. Serum was from blood withdrawn from the heart under terminal ether anesthesia. Dust was taken from the ventilation grill of a room housing only specific pathogen-free S-D/G rats. Sawdust and diet (PRD diet; Labsure, Poole, U. K.) used for extracts had not been in contact with animals.

Preparation of extracts

Animal material was stored at -20° C before extraction.

Solid materials (pelt, feces, etc.) were dried by lyophilization and defatted in diethyl ether (1/10 w/v) for 24 hr.

The dried material was extracted for 72 hr in Coca's solution, pH 7.2 (1/10 w/v).³⁷ Such extracts and undiluted liquid materials (urine, saliva, serum) were clarified by centrifugation and sterilized by filtration (0.45 μ m). Dialysis was carried out for 3 days at 4° C against several changes of a vast excess of 0.05M ammonium hydrogen carbonate (pH 7.85) before freeze drying.

TABLE I. Quantities of rat-derived material extracted and dry weight yields

Source material	Unit	Sex of rat	No. of "units" extracted	Mean volume or dry weight per unit before extraction	Mean dry weight of extract per unit (mg)	Dry weight of extract relative to starting material
Urine	Collection per rat (24-hr)	M	152	6.1 ml	21	3.4 mg/ml
		F	88	8.6 ml	13	1.5 mg/ml
Saliva	Period of collection per rat	M	32	3.0 ml	15	4.9 mg/ml
		F	34	2.8 ml	15	5.4 mg/ml
Fur	Whole body of dead rat	M	4	5.2 gm	8.2	1.6 mg/gm
		F	4	2.8 gm	4.1	1.5 mg/gm
Epithelia	Whole body of dead rat	M	4	2.8 gm	41	15 mg/gm
		F	4	1.7 gm	26	15 mg/gm
Shaved pelt	Whole body of dead rat	M	4	49 gm	550	11 mg/gm
		F	4	27 gm	410	15 mg/gm

TABLE II. Percent of protein (w/w) in extracts derived from or associated with male rats

Extract	% Protein
Saliva	25
Urine	25
Fur	13
Epithelia	31
Shaved pelt	65
Whole pelt	52
Serum	51.5
Feces	14.5
Dust	10
Sawdust	14
Diet	16

Protein determination

Protein concentrations were determined by the dye-binding method of Bradford³⁸ with the use of a bovine albumin standard (Bio-Rad Laboratories, Richmond, Calif.).

Skin tests

Skin-prick tests were performed with extracts at 0.01, 0.1, and 1 mg/ml dry weight in Coca's solution/glycerol (1/1) heated at 56° C for 30 min and filtered (0.45 µm).

Initially extracts were tested at 0.01 mg/ml, and only if there was no positive reaction was the concentration increased to 0.1 or 1 mg/ml⁻¹. A wheal of 3 mm diameter or greater at 15 min was arbitrarily considered positive. Wheal area was calculated from measurements of mean wheal diameter.

Sera

Sera was obtained from rat-allergic subjects who associated symptoms of asthma and rhinitis with exposure to rats. All had been exposed to other laboratory animals in addition, and most were skin-prick test positive to several species.³⁵

Control subjects were laboratory workers who recognized no allergic symptoms as being caused by rats, although about one-third of this group were atopic as determined by skin sensitivity to one or more common inhalant allergens.³⁹

RAST

RAST for specific IgE was developed according to a method previously described⁴⁰ with extracts covalently coupled (10 mg/gm by dry weight) to cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden).

Optimal conditions for RAST were determined by varying the concentrations of patients' sera, APC, and the radiolabeled anti-IgE. Sensitivity was found to be greater with 50 µl undiluted patients' sera and with 50 µl ¹²⁵I-labeled anti-IgE (Pharmacia). APC was in excess at concentrations higher than about 2.5% (Fig. 1).

With all of the allergen extracts tested, 100 µl of 5% APC was incubated with 50 µl of patients' sera in polystyrene tubes (LP4; Luckham, Burgess Hill, Sussex, U. K.) and was shaken for 16 hr. After three washes with normal saline containing 5% Tween 20, 50 µl ¹²⁵I-anti-IgE was added, and tubes were shaken for a further 16 hr. After the tubes were washed as before, they were counted for 2 min in a gamma counter. All sera were assayed in duplicate.

Results were expressed as the percentage of the total counts added that bound to the antibody APC.

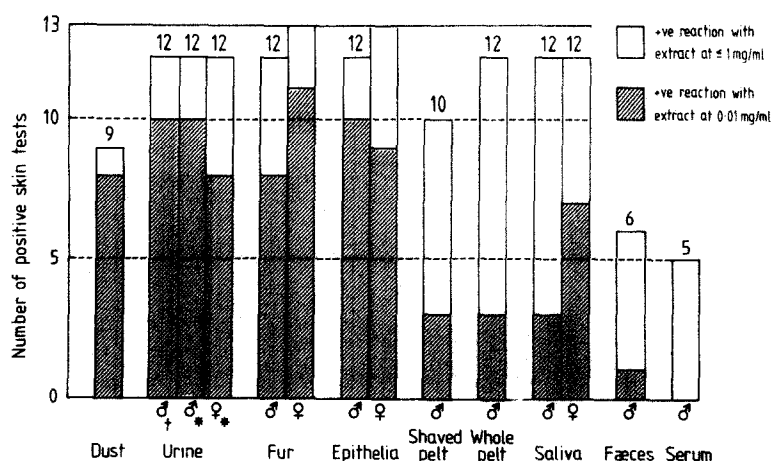


FIG. 2. Total numbers of positive skin-prick test reactions (≥ 3 mm) elicited by each extract at a concentration of 0.01 mg/ml^{-1} and at 1 mg/ml^{-1} in 13 rat-sensitive patients.

*Collected by metabolic cage.

†Collected directly.

TABLE III. Range of skin-test wheal areas and median and mean values for rat-derived extracts tested at 0.01 mg/ml^{-1} in 13 rat-sensitive patients

Extracts	Sex	No. of patients with wheal area (mm^2) in the range						Wheal (mm^2) area	
		0	0.1 to 5.0	5.1 to 10.0	10.1 to 15.0	15.1 to 20.0	>20.0	Median	Mean
Dust		0	4	5	3	0	1	7.1	11.9
Urine*	M	1	2	3	2	5	0	12.6	10.2
Urine†	M	0	3	4	3	2	1	9.6	11.6
Urine†	F	1	4	5	2	1	0	7.1	7.1
Fur	M	0	5	4	3	1	0	7.1	8.5
Fur	F	0	2	7	2	2	0	9.6	10.0
Epithelia	M	1	2	7	2	1	0	7.1	7.8
Epithelia	F	0	3	5	4	1	0	7.1	8.6
Shaved pelt	M	5	5	3	0	0	0	0.8	2.7
Whole pelt	M	2	8	3	0	0	0	3.1	3.5
Saliva	M	1	7	3	1	0	1	4.9	6.6
Saliva	F	2	1	7	1	2	0	7.1	8.4
Faeces	M	7	5	1	0	0	0	0	1.9
Serum	M	8	5	0	0	0	0	0	1.2

*Collected by bladder massage.

†Collected with metabolic cages.

RAST inhibition

Fifty microliters of pooled allergic or control sera was incubated in duplicate with $100 \mu\text{l}$ of extract in a concentration range from 0.025 to 100 gm/ml dry weight in phosphate-buffered saline. After shaking the concentration in capped tubes for 6 hr at room temperature, $100 \mu\text{l}$ of 5% APC was added, and the RAST procedure was carried out as described.

Percent inhibition was calculated according to the formula:

$$\% \text{ inhibition} = \frac{(A-C) - (a-c)}{A-C} \times 100\%$$

where A, C, a, and c represent the counts obtained for uninhibited allergic serum, uninhibited control serum, inhibited allergic serum, and inhibited control serum, respectively.

RESULTS

Yields of freeze-dried extracts

The dry weight yields are demonstrated for extracts of rat urine, saliva, fur, epithelia, and shaved pelt and are expressed in terms of the amount of starting material (Table I). Variability in the volumes, weights, and concentrations of source materials collected from

TABLE IV. RAST for IgE against extracts of male rat epithelia, fur, saliva, urine, and feces and dust from a rat room

Rat-allergic patients	Mean percent binding					
	Epithelia	Fur	Saliva	Urine	Feces	Dust
S. L.	25.9	27.3	22.2	30.1	12.0	23.8
S. K.	24.7	20.9	21.0	27.1	16.4	29.0
A. S.	24.4	19.7	20.7	27.5	7.0	24.4
L. L.	23.7	25.1	25.8	22.6	14.2	26.3
D. C.	23.5	23.4	23.7	24.6	6.3	29.7
C. A.	23.0	22.2	24.7	23.8	10.4	26.3
G. H.	21.1	22.7	18.4	29.0	6.7	18.5
A. C.	21.0	23.8	24.4	28.0	1.6	26.1
D. A.	20.4	14.3	17.5	24.3	7.1	16.4
P. W.	17.9	21.2	18.1	25.0	0.9	19.6
P. K.	17.5	19.8	16.8	18.0	6.8	16.8
G. S.	10.8	10.5	11.1	12.8	3.5	17.4
J. L.	5.4	2.4	3.8	8.1	0.9	5.2
A. B.	4.7	9.4	3.8	3.4	2.9	2.5
7 control sera (mean)	1.8	1.1	1.2	1.7	1.2	0.51
Upper 95% confidence limits	2.3	1.3	1.5	2.4	1.5	1.0
Cord serum	1.2	0.8	1.2	1.4	1.2	0.4

TABLE V. Significance of Spearman's rank correlation coefficient comparing RAST for IgE against six different rat-derived extracts

Rat extract	Epithelia	Fur	Saliva	Urine	Feces
Dust	<0.01	<0.01	<0.001	<0.05	<0.05
Feces	<0.01	NS	<0.025	NS	—
Urine	<0.01	<0.01	<0.05	—	—
Saliva	<0.01	<0.001	—	—	—
Fur	<0.01	—	—	—	—

rats was compensated for by the extraction of relatively large quantities.

Protein determination

The protein contents as percentage dry weight of freeze-dried extracts are illustrated in Table II.

Skin prick tests

Positive reactions were elicited in 12 of 13 patients who associated asthma and rhinitis to rat exposure when they were tested with rat urine, fur, epithelia, and saliva extracts (all irrespective of sex or rat) at concentrations of 1 mg/ml or less (Fig. 2). The remaining patient reacted only to fur and epithelia. In addition, 12 patients reacted to extracts of whole pelt, 10 to shaved pelt, 10 to dust collected from a rat room, six to feces, five to serum, and one to sawdust at maximum concentrations or less.

There were no positive reactions to rat diet or sawdust. Skin-test wheal areas were calculated for 13 rat-sensitive patients who reacted at extract concentrations of 0.01 mg/ml. For each extract the range of skin-test reactions is indicated as well as median and mean values (Table III). No positive skin-prick tests were obtained in 10 asymptomatic control subjects with the same range of extracts at the top concentration, although five subjects of this group were regularly exposed to rats, and three subjects were atopic as determined by skin sensitivity to one or more common inhalant allergens.³⁹

RAST

Results of RAST for serum IgE are demonstrated with the following male, rat-derived extracts coupled to Sepharose particles: epithelial scrapings, fur, saliva, urine, feces and dust from a rat room (Table IV).

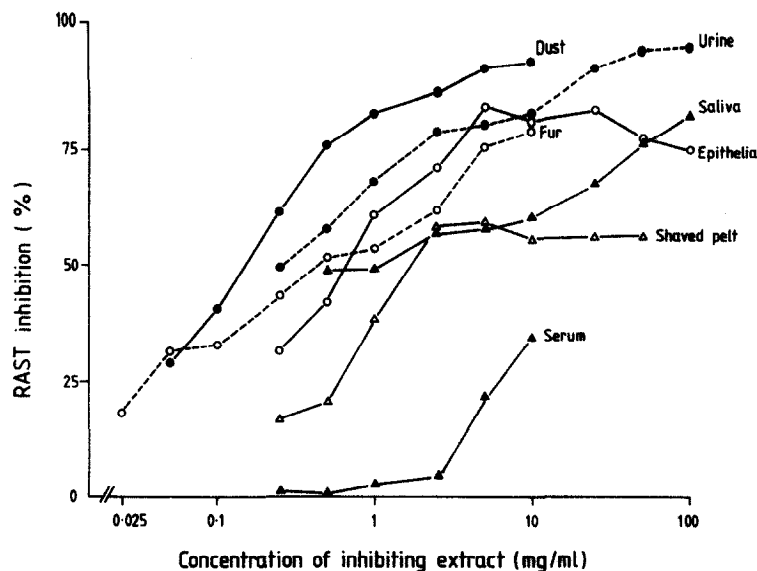


FIG. 3. Inhibition of IgE RAST for dust from a rat room with various rat-derived extracts.

All 14 patients with asthma and rhinitis after exposure to rats and with positive skin tests to rat extracts had higher levels of specific IgE than the nonrat-allergic subjects. Only in the RAST for rat feces did allergic patients have levels within the range found for control sera, although even in this case, 12 of the 14 had raised IgE against fecal allergen. Lower percent binding for control sera in the RAST for rat dust may be attributed to higher counts added (73,000 counts per 2 min as opposed to 25,000 counts per 2 min for the other five assays).

A normal distribution was assumed for values of RAST binding for control sera when 95% confidence limits were calculated. RAST data for rat-allergic subjects, however, were not normally distributed, and in investigating the extent of correlation between source materials, nonparametric statistics were used. RAST scores were ranked, and rankings with different extracts were compared by calculating Spearman's coefficient of rank correlation.⁴¹ Significant relationships ($p < 0.05$) were found between relative RAST values by use of all these six source materials, except when the RAST for feces was compared with that for fur or urine (Table V).

RAST inhibition

Inhibition of a RAST for IgE to dust from a rat room was achieved with many extracts by use of pooled sera from 12 rat-allergic subjects (Fig. 3). In the assay illustrated, mean uninhibited RAST binding of rat dust by the allergic serum pool was 25.2% and 0.9% by the control pool of total counts added (39,700 counts per 2 min). There was no inhibition with pooled serum from nonrat-sensitive subjects.

Relative potencies were similar when the assay was

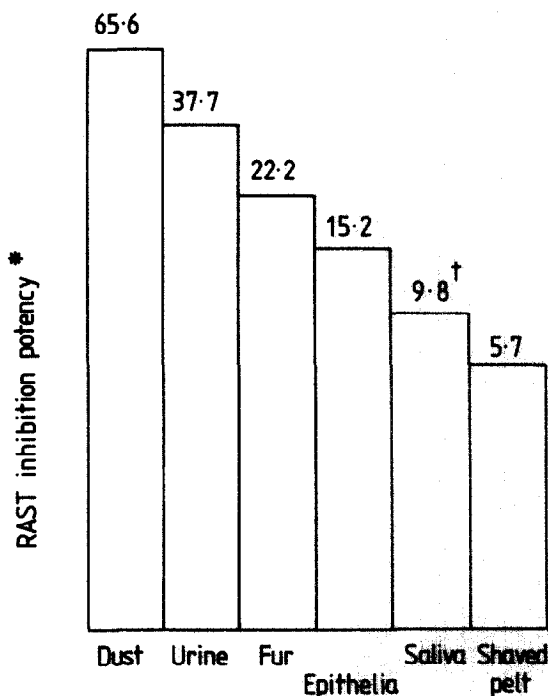


FIG. 4. Relative potencies of some rat-derived extracts in IgE RAST inhibition of dust from a rat room.

*Reciprocal of the amount of extract in milligrams (dry weight) required to achieve 50% inhibition of IgE RAST scores to dust from a rat room (logarithmic scale).

†Estimated by extrapolation.

carried out separately by use of extracts derived from male or female rats. Most reactive, apart from dust itself, were urine, fur, epithelia, and saliva. Shaved pelt, feces, and serum were less potent, whereas dust from a guinea pig room, house dust, and sawdust all caused negligible inhibition of rat-allergic sera.

The dry weight of extract required to inhibit IgE RAST scores to dust by 50% was determined for those extracts for which this degree of inhibition was achieved over the concentration range tested. The reciprocals of these figures were used as a measure of relative potency (Fig. 4).

The slopes of logit-log transformed RAST-inhibition data were analyzed for the extracts of dust, urine, fur, epithelia, saliva, and shaved pelt. The procedure involved taking the logit of the percent inhibition, the logarithm of the concentration of inhibiting extract, and a common slope was fitted for all extracts as described by Armitage.⁴² Analysis of all data points revealed a significant difference ($p < 0.05$) in the slopes. When data for shaved pelt, or shaved pelt and saliva, or shaved pelt and epithelia were excluded from calculations so that only those extracts with slopes that were the most similar were compared, the divergence from parallelism was still significant ($p < 0.05$).

Although a sigmoidal RAST inhibition curve ought to be transformed to a straight line along its entire length by use of these statistical procedures, a greater scatter of points would be expected at the extremities. Unless large numbers of measurements were taken, this could alter the gradient of the line. The central portion of a RAST-inhibition curve must, therefore, represent the most suitable region for estimation of slope. In these investigations, however, no loss of linearity was observed at either end of transformed RAST-inhibition curves, and restricting analysis of slope to data within the 20% to 80% inhibition range did not reveal parallelism.

DISCUSSION

A broad range of extracts derived from rats were found to be allergenic. In fact, for almost every rat-sensitive subject in this study, RAST and skin tests were positive with extracts of fur, epithelia, urine, saliva (all irrespective of the sex of source animal), and with dust from a rat room. Possibly some of the other rat extracts, such as feces or serum, would have provoked more positive skin-test reactions had they been tested at higher concentrations.

Certain differences in the allergenic composition of rat source materials were revealed by analysis of the slopes of RAST-inhibition curves. Parallelism of inhibition curves in radioimmunoassay for a single protein has been taken as an indication of close antigenic identity.^{43, 44} In RAST inhibition with whole extracts containing several allergens, however, the situation is more complex. The inhibition curve for each of the constituent allergens is likely to be different, and the inhibition curve of the whole extract will be determined not only by the extent of antigenic cross-reac-

tivity between the individual components of different extracts but also by the relative amounts of each allergen in the inhibiting extract.

Although similar slopes of logit-log transformed RAST-inhibition data have been reported where extracts were of essentially the same material (e.g., different commercial preparations of ragweed pollen⁴⁵), this was not observed when the different rat-derived extracts were added to a RAST for IgE specific for dust from a rat room. Presumably, the deviation from parallelism has resulted from there being different proportions of the constituent allergens in the extracts.

Similar allergenic components were evidently present in all the more potent rat source materials. There was a close correlation between RAST results with extracts of rat fur, epithelia, saliva, urine, and dust. Unlike analysis of inhibition curves, this should provide a strictly qualitative measure of cross-allergenicity, as the allergen-particle complex was added in excess to the RAST procedure (Fig. 1). Moreover, it was observed that the skin-test sensitivity of rat-sensitive patients was in general to several extracts, and extracts of urine, fur, epithelia, and saliva as well as dust from a rat room were each found to be capable of inhibiting the RAST for IgE to dust by more than 75%. Furthermore, extensive antigenic and allergenic similarities have been demonstrated between rat fur, urine, saliva, and other extracts by use of quantitative immunoelectrophoretic techniques.^{34, 35}

Many of the source materials used would, in the natural state, rapidly become contaminated with each other, and it appears likely that dust in a rat room would contain to a greater or lesser extent all of the materials from which extracts have been made. Precautions were taken during collection so that the saliva, feces, serum, sawdust, and diet were uncontaminated. Although urine collected by metabolic cage may have become contaminated with other materials, the skin-test activity was similar to that obtained directly by bladder massage and little difference in antigenic composition has been demonstrated by immunoelectrophoretic analysis of these extracts.³⁴

There were some clear differences in skin prick-test reactivity between extracts from different sources when the extracts were compared on a dry weight basis. Sex differences for the same rat source material were more equivocal. Although the extracts of urine from male rats proved more reactive than that from female rats, the reverse was the case with fur, epithelia, and saliva. It is difficult to assess the significance of these differences on account of the highly irregular distribution of skin test data, and batch-to-batch variation of extract potency was not investigated. Nevertheless, neither the measurements of allergenic activity reported here nor immunoelectro-

phoretic analysis³⁴ have indicated that the sex of the source animal is of overriding importance when allergenic extracts are prepared.

For most rat-source materials collected, there was a tendency for a higher dry weight yield of extract to be obtained from male animals than from female animals. These differences, however, were of an order that would be expected bearing in mind the larger size of male rats of the same age.

It may be assumed that dust from a rat room represents the material that causes asthmatic reactions when the material is inhaled by rat-allergic subjects. IgE RAST inhibition of the dust thus provides a guide to the relevance of individual source materials. The pattern of activity that has emerged from these RAST-inhibition experiments was similar to that which was obtained by skin-prick tests.

Rat bedding and diet were not found to be of importance as a source of allergens for the rat-sensitive patient studied, and there was insignificant cross-reaction between allergens present in dust from a rat room and either house dust or dust from a room housing guinea pigs. Feces, although containing some allergenic material, were much less potent than most of the other extracts. It is improbable that feces are a major source of allergenic material. Another of the less potent extracts, rat serum, is unlikely to act *per se* as a source of allergenic material, although some serum proteins have been detected in all of the rat-derived extracts.³⁴ The relative lack of allergenic activity of rat serum confirms previous work that demonstrated serum preparations to be less effective in skin tests of rat-sensitive patients than whole pelt¹⁵ and urine.¹⁶

Three remaining sources of rat allergens are the skin, urine, and saliva. Caution must be exercised in deducing the principal sources of rat allergens from measurements of potency on a dry weight basis, especially in view of the cross-allergenicity that has been demonstrated. Relating potency to protein content is another method that has been used as a basis for comparison of allergen extracts, but this makes the assumption that the carbohydrate constituents are unimportant. In this study it is unlikely that the general pattern of results would have been greatly altered by comparing extracts in this way. Protein determinations of the more potent allergen extracts did not differ by more than twofold, and it was found that extracts with the highest protein contents such as shaved pelt and serum were also among the least allergenic.

Considerations of the relative amounts of each source material with which rat handlers come into contact must be of much greater importance. These parameters, however, are exceedingly difficult to measure and must depend on such factors as the method

of animal husbandry and the nature of an individual's exposure to rats. Nevertheless, extracts of rat fur or epithelia, urine, and saliva all proved most effective in immunologic tests, and these materials are all likely to be abundant in the environment of rats.

Newman Taylor¹⁶ and colleagues have drawn attention to the potential for dust contaminated with urine to act as a source of allergens. The confinement of rats in small cages could also result in contamination of the pelt with urine.

Saliva, despite the relative neglect of this source material in allergy to rats, may possibly be a more important source of allergens in the fur or dander. Grooming represents a conspicuous part of rat behavior⁴⁶ and particularly at night when these animals are most active and when the rate of secretion of saliva is at a maximum.⁴⁷

In these experiments, where extracts were produced from three layers of pelt, it was the outer layer ("fur") that proved most allergenic. Where contamination with saliva or urine was minimal ("shaved pelt"), the extract was considerably less potent when it was compared according to the dry weight. Moreover, immunoelectrophoretic analysis has revealed that many of the antigenic constituents of rat fur are not present in the extract of shaved pelt.³⁴

The possibility that potent skin-derived allergens are selectively concentrated on the outermost layer of the pelt cannot be excluded. Sebaceous glands in rat skin have been the subject of some studies,⁴⁸⁻⁵⁰ and it is known that the oily secretions of these glands are responsible for the water-barrier properties of fur.⁵¹ The insolubility of sebum in aqueous solutions, however, can hardly be considered as being compatible with allergenic activity. The selective excretion of allergenic components in sweat may also be discounted since sweat glands are not present in the hairy skin of rats but are restricted to the footpads.⁵²

The dry weight yield of extract from an animal was found to be higher for full thickness skin than for shaved fur, but whole pelt must be considered as inferior to fur or epithelia as an extract for use in diagnosis, and especially in immunotherapy, as it contains greater amounts of nonallergenic or only weak allergenic material. Saliva and urine probably represent more important primary sources of allergens than the skin.

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