

Allergy to rats: Quantitative immunoelectrophoretic studies of rat dust as a source of inhalant allergen

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The antigenic composition of an extract of rat dust, as a source of aeroallergens for rat-sensitive individuals, has been investigated and compared to the antigenic composition of rat saliva and urine. Of four main antigenic peaks identified by crossed immunoelectrophoresis, one antigenic peak (Ag 4) was demonstrated to be antigenically closely related to and with similar molecular weight (approximately 22 kd) and isoelectric point values as urinary prealbumin, already recognized as a major rat allergen. Ag 4 was present in all dusts studied and was also identified as a minor component of saliva. However, no component with the same electrophoretic mobility or physicochemical characteristics of the α_2 -euglobulin of male rat urine that shares partial identity with the prealbumin was detected, even in dust collected from a male rat room. A second high molecular weight (>200 kd) component, Ag 1, present in most of the dust extracts, could not be detected in either urine or saliva. Crossed immunoelectrophoresis and skin prick tests confirmed the allergenicity of both these antigens. Analysis of an air filter sample taken within a male rat room revealed significant amounts of the "prealbumin" component, and a monospecific antiserum to this component was used to quantitate levels in dusts collected from various locations. These findings suggest that a major inhalant allergen present in rat dust is closely related to urinary prealbumin but that this and other allergenic components may not be derived predominantly from rat urine or saliva but possibly from secretions originating from the skin of the animals and present in the fur. (J ALLERGY CLIN IMMUNOL 1987;80:243-51.)

Recent studies of rat-allergen extracts have demonstrated that the protein present in rat urine, the prealbumin (MW 20 to 21 kd, pI 4.5 and 4.6) and the partially cross-reacting α_2 -euglobulin (MW 16 to 18 kd, pI 5.4, 5.6, and 5.8), are highly reactive in rat-allergic individuals.¹⁻³ However, the dusts collected from exhaust filters of animal-housing areas and by aerobiologic methods may be regarded as more representative of the inhaled aeroallergens causing sensitization of exposed subjects. Such dusts are likely

Abbreviations used

kd:	Kilodaltons
XIE:	Crossed immunoelectrophoresis
XRIE:	Crossed radioimmunoelectrophoresis
IG:	Intermediate gel
pI:	Isoelectric point

to contain particles derived from the hair and dander of the animals, as well as diet and bedding, all of which could be contaminated with saliva, urine, and feces. Furthermore, some components may be subject to enzymatic degradation after their excretion/deposition. Therefore, comparisons of the antigenicity of the dust to those of single source materials may be used to identify the actual origin(s) of the dust components. It is also important with regard to the development of relevant tests for aeroallergen quantification that the antigenic/allergenic nature of the airborne material is known.

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The aim of the present study, therefore, was to characterize by various quantitative electrophoretic techniques the antigenic components of rat dust extract and compare these components to other previously studied rat-source materials,^{1, 2, 4} in particular, urine and saliva.

MATERIAL AND METHODS

Dust samples were collected from extract filters and electrostatic air cleaners of a number of male and female rat rooms at different animal research facilities (thereby included various rat strains). The dust used most extensively in this study (F9) came from the outlet fan and cover assembly of a male rat room that had not been cleaned (generally once per annum) for 11 months. The air sample was taken from the same rat room by a Staplex (Staplex Co., Brooklyn, N. Y.) high-volume air sampler, which collects particles to 0.2 μm in diameter, and run for 4.3 hours at 1800 L/min (i.e., 360 m³ of air sampled with 49 mg of dry weight collected).

Rat urine from 3- to 4-month-old male rats (Wistar) was collected daily and frozen, and rat saliva was obtained by stimulation with pilocarpine nitrate with ketamine hydrochloride anesthesia.

The dust extracts were prepared by extraction (48 hours) in Coca's solution (1:10 w/v). Such extracts or liquid source materials were clarified by centrifugation, membrane filtered (0.45 μm), dialyzed extensively at 4° C against 0.05 mol/L NH_4HCO_3 (to avoid precipitation of any α_2 -euglobulin component, if any were present), and freeze-dried.

Rabbit antisera

Rabbit antisera were produced in rabbits (New Zealand white) by repeated subcutaneous and intramuscular injections of the relevant antigen emulsified with Freund's incomplete adjuvant. Initially, 25 mg of antigen was administered, and thereafter, the rabbits were boosted with a saline solution (2 mg/ml) until a satisfactory response was obtained, as determined in XIE tests. Polyspecific antiserum pools were then prepared with high-titer sera from two or three rabbits per antigen.

Monospecific antiserum to the prealbumin (fraction 3) of rat urine¹ was also similarly produced. Antirat serum was purchased from Dakopatts A/S (Dako-immunoglobulins, Copenhagen, Denmark).

Human sera

Sera were obtained from rat-allergic subjects who associated symptoms of asthma and rhinitis with exposure to rats and who were positive in skin prick tests and by RAST to rat-urine antigen.²

Skin tests

Skin prick tests were carried out on five rat-allergic patients with the original dust extract, the high-molecular-weight and low-molecular-weight fractions, at 1 mg/ml.

Gel filtration

Gel filtration of rat dust extract (F9, 200 mg/2 ml) was carried out at room temperature on a Sephacryl S200 column (90 by 2.6 cm) equilibrated in NH_4HCO_3 (0.05 mol/L, pH 8) at a flow rate of 30 ml/hr. The eluate was monitored at 280 nm (Uvicord II, LKB, Bromma, Sweden) and collected in 4 ml fractions. Molecular weight calibration of the column was carried out with blue dextran 2000 (BDx), albumin (67 kd), ovalbumin (43 kd), chymotrypsin A (25 kd), cytochrome C (12.5 kd), and vitamin B₁₂ (1.4 kd).

XIE/XRIE

These tests were performed as previously described.^{3, 4} Briefly, tests were carried out on 8.2 cm² glass slides in 1.2 mm thick agarose gels (1% w/v electran agarose 15, BDH, Poole, U. K.) containing 0.025 mol/L, pH 8.65, of veronal buffer. Antigen was electrophoresed in the first dimension at 4.5 V/cm for 80 minutes with second dimension electrophoresis carried out at 1.5 V/cm overnight. In the figures, the anode is to the *right* for the first and to the *top* for the second dimension electrophoresis.

An IG (5 by 1 cm) containing antirat serum (15 $\mu\text{l}/\text{cm}^2$ of gel) was placed between the electrophoresed extract and the main antiserum-containing gel area (5 by 3.5 cm) with 15 μl of antisera per square centimeter of gel. Also, for some tests, a second similar-sized IG was interposed between these two gel areas.

For comparisons of antigenic identity between components present in some extracts, pairs of XIE tests were performed to determine the alteration in XIE profile of one extract by addition of a second.

For XRIE,³ the plates were washed and dried (cold air) and then overlaid, first with patient's (or control) serum, and then, after washing, with ¹²⁵I-labeled anti-IgE (Phadebas RAST isotope, Pharmacia Diagnostics, Uppsala, Sweden) at approximately 30,000 cpm per plate. The washed and dried plates were then exposed to x-ray film (DEF-2, Eastman Kodak Ltd., Rochester, N. Y.) for 5 to 7 days; after which the filters were developed according to manufacturer's instructions, and the glass slide stained for protein in 0.5% Coomassie brilliant blue R250 (Sigma Chemical Co., St. Louis, Mo.).

Rocket immunoelectrophoresis

For estimation of the content of the urinary prealbumin component in the dust samples, extracts (200 mg of dust per milliliter of saline) were electrophoresed directly into gels containing the monospecific antiserum to this component, and their peak heights were measured. Electrophoretic conditions were as for second dimension XIE. A standard curve was obtained with dilutions of a purified ($\geq 95\%$) prealbumin fraction.

RESULTS

Antigenic characterization of rat dust

Relationship of dust with urine antigens. From the XIE profiles of rat dust against antirat dust

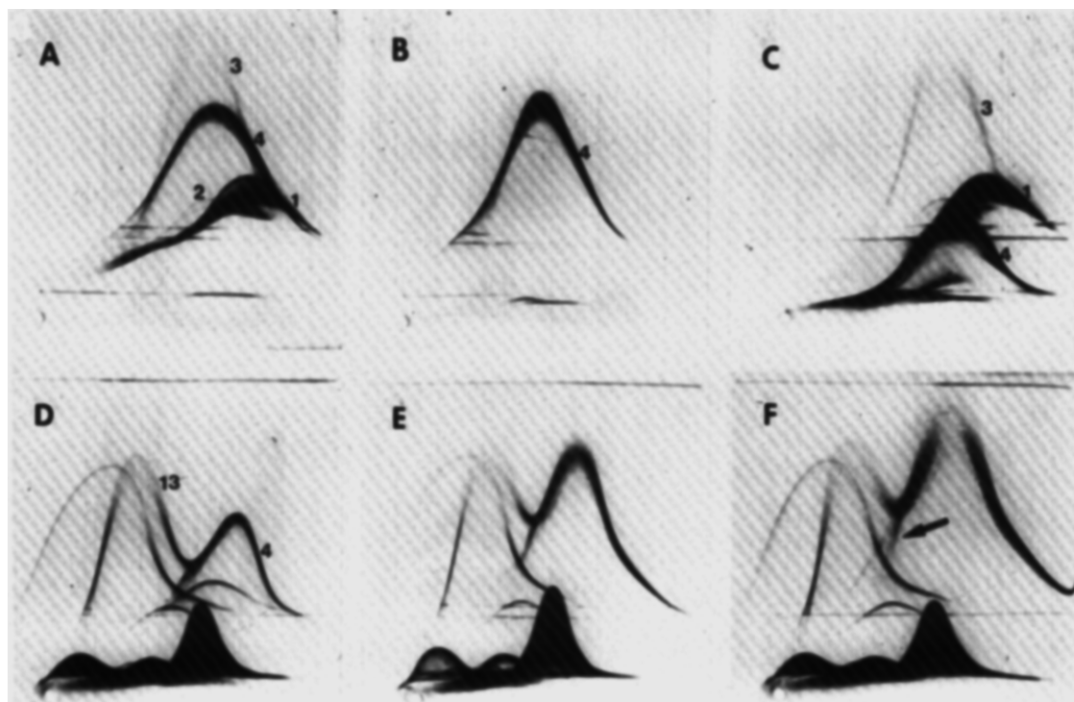


FIG. 1. XIE tests demonstrating the antigenic relationship of rat dust (F9) to male rat urine. Rat dust (30 μ g) against **A**, antirat dust, and **B**, antirat urine, both with IG incorporating antirat serum, and **C**, against antirat dust but with the antirat urine in the IG. Although four main antigenic components are present in the rat dust (**A**), only a single strong antigen peak is revealed by the antiurine (**B**), and this is identified as Ag 4 by its absorption (**C**). Rat urine (15 μ g) against antirat urine with IG of antirat serum, **D**, and control, **E**, with 16 μ g, and **F**, with 30 μ g of rat dust added to the rat urine during the first dimension electrophoresis. Addition of the dust caused increases in the height of the urinary prealbumin peak in **E** and **F** compared to **D**, but no change in that of the urinary α_2 -euglobulin peak 13. The reaction of partial identity between these components is maintained in **E** but slight forking occurred (arrow) with the higher amount of dust added in **F**.

(Fig. 1, **A**) and antiurine (Fig. 1, **B**), it was evident that there were antigens other than urinary components present in the dust. In particular, it was noted that, although the dust was obtained from a male rat room, the main component recognized by the urine antiserum did not demonstrate the characteristic pattern of reactivity of male urine (compare with Fig. 1, **D**), i.e., the major α_2 -euglobulin peak (Ag 13) with its partial identity to the prealbumin component (Ag 4). (The numbering of the antigenic peaks is in accordance with previous studies of rat allergenic extracts,^{3,4} such that the urinary prealbumin is Ag 4 and the α_2 -euglobulin is Ag 13.) By incorporation of the antiurine in an IG in the dust/antidust profile (Fig. 1, **C**), the component with antigenic identity to the urinary component was identified, by its absorption, as the symmetrical peak of electrophoretic mobility slightly slower than the urinary prealbumin (i.e., approximately serum albumin). There was no effect on Ag 1.

When amounts of the dust extract were added to rat urine in a rat urine/antirat urine XIE (Fig. 1, **E** and **F**), there was no alteration in the peak height of the α_2 -euglobulin (compare with Fig. 1, **D**); however, there were increases in the area of the prealbumin with the peak maximum, demonstrating a slower electrophoretic mobility, and the reaction of partial identity between the two components was maintained, although with the higher amount of dust added (Fig. 1, **F**), some forking of the "spur" was noted.

To study further the antigenic interrelationships between the dust and urine components, urine was tested against the dust antiserum (Fig. 2, **A**). As expected from their partial identity, both the prealbumin and α_2 -euglobulin components were recognized. With the antidust in a second IG of the urine/antiurine (Fig. 2, **B** and **C**), although both peaks demonstrated absorption, there was proportionally greater reduction in the prealbumin compared to the α_2 -euglobulin.

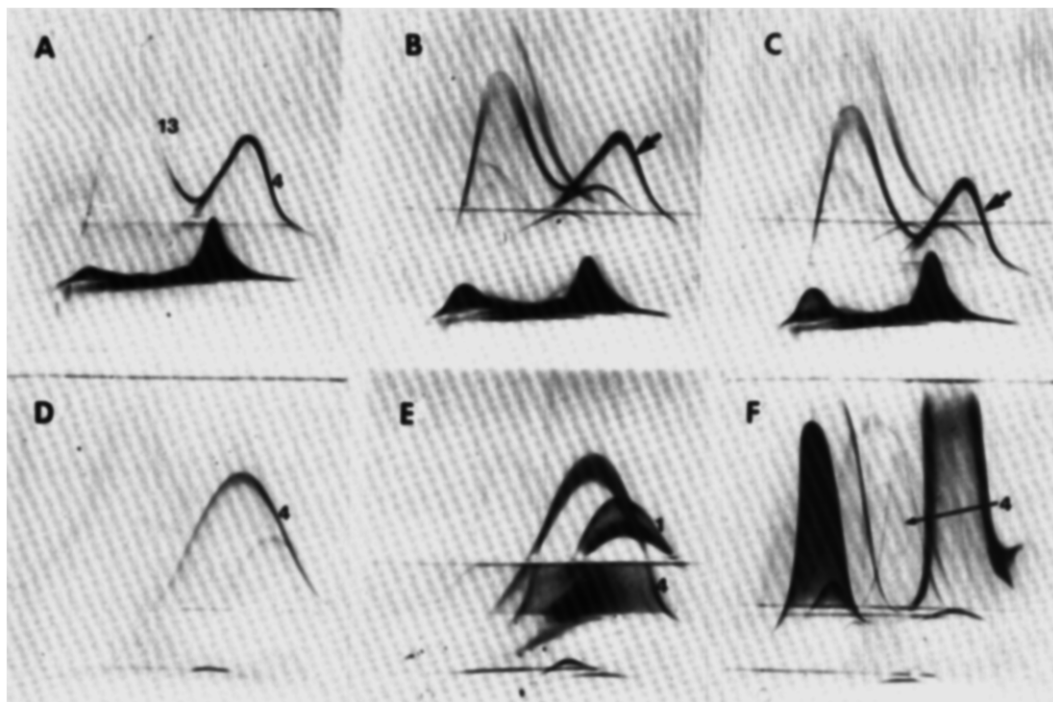


FIG. 2. XIE tests demonstrating antigenic relationships of rat dust to rat urine and saliva. **A**, Rat urine (15 µg) against antirat dust with IG of antirat serum. **B-C**, Rat urine (15 µg) against antirat urine with first IG antirat serum, and with second IG (**B**) control and (**C**) containing antirat dust. The marked absorption of the urinary prealbumin peak (Ag 4) is indicated. **D**, Rat dust (15 µg) against antirat saliva. **E**, The antirat saliva in a second IG of rat dust against antirat dust (compare with Fig. 1, A). **F**, Rat saliva (15 µg) against antirat saliva. The peak identified by incorporating a monospecific antiserum in an IG (not illustrated) as prealbumin, i.e., Ag 4, is indicated.

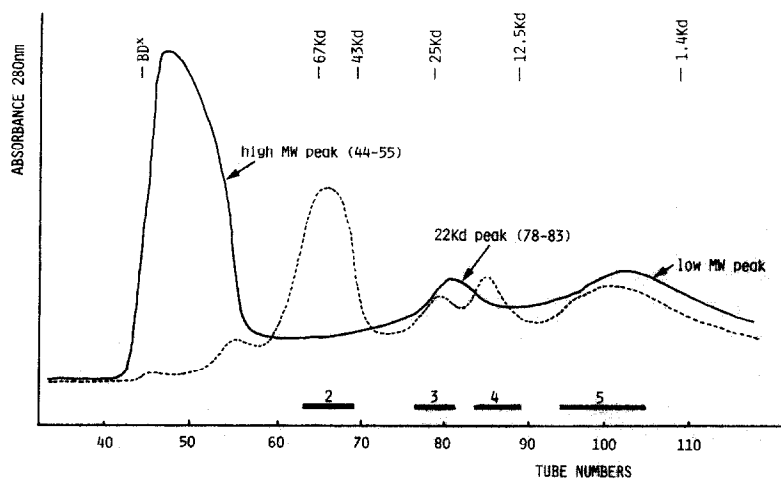


FIG. 3. Optical density profile of Sephacryl S200 gel filtration of rat dust F9 (—) compared to that of male rat urine (---). The three dust peaks and the urine fractions 2 to 5, as in an earlier article,¹ are indicated.

These results indicate that for the dust component, Ag 4, there was closer antigenic identity to the urinary prealbumin than to α_2 -euglobulin.

Relationship of dust and saliva antigens.

Fig. 2, **D**, illustrates the XIE profile of the dust with antiserum to saliva, revealing again one strongly stained peak (identified as prealbumin, Ag 4, by incorporating a specific antiserum in an IG, not illus-

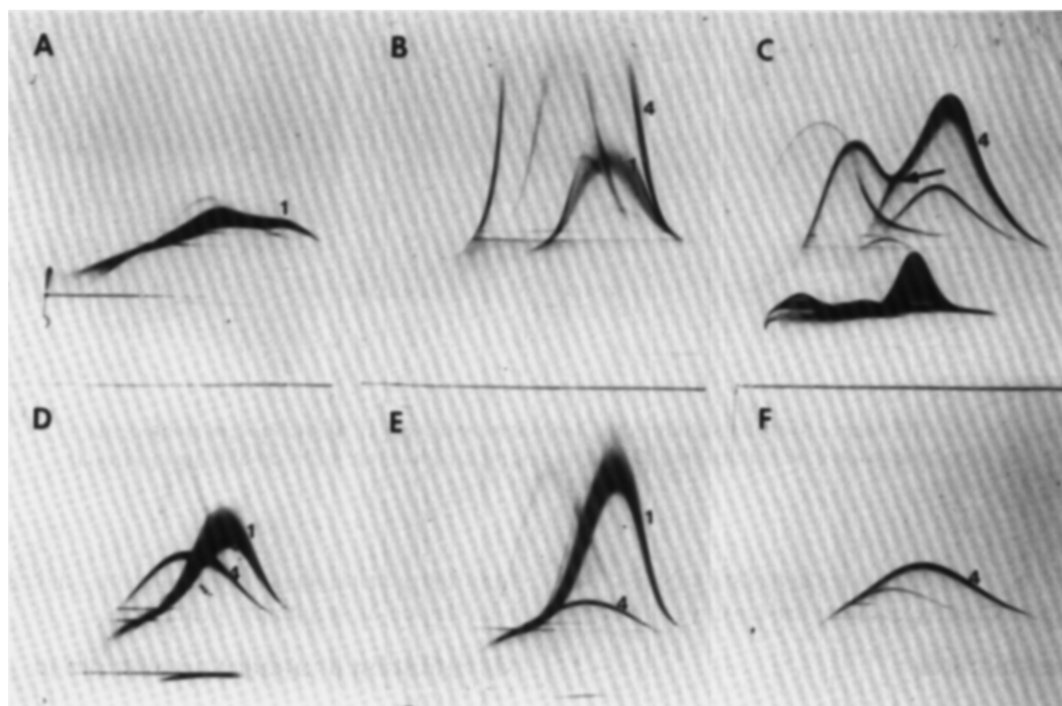


FIG. 4. XIE tests of rat dust fractions and further dust extracts. **A-B,** Fractions of rat dust (30 µg) from Sephacryl S200 gel filtration. **A,** High-molecular-weight fraction (tubes 44 to 55). **B,** 22 kd fraction (tubes 78 to 83) against antidust. **C,** 22 kd fraction (5 µg) added to rat urine (15 µg) against antirat urine demonstrating the large increase in the urinary prealbumin component (compare with Fig. 1, D) with the reaction of partial identity remaining. **D-F.** Rat dusts against antirat dust. **D,** F8 (30 µg). **E,** F22 B (30 µg). **F,** Staplex SI (300 µg).

trated), two weak antigen peaks, but no Ag 1. By incorporation of the antisaliva in a second IG, all the peaks demonstrated some absorption, but in particular Ag 4, i.e., the prealbumin peak (Fig. 2, E). The homologous rat saliva/antisaliva profile (Fig. 2, F) illustrates the rather slower mobility of the component analogous to the prealbumin and the numerous other components that are not present in the dust extract, i.e., absent from the rat dust/antisaliva profile (Fig. 2, D).

Gel filtration

The optical density profile of Sephacryl S200 fractionation of F9 dust, compared to that of male rat urine,¹ is illustrated in Fig. 3. An initial high-molecular-weight peak (tubes 44 to 55) appeared after the void volume, a second smaller peak (tubes 78 to 83, molecular weight 22 kd) eluted in a similar position to that of the urinary prealbumin (urine fraction 3), and a third very low-molecular-weight peak (compare with fraction 5 of urine) that consisted of pigment and odor-containing components. No peak corresponding to serum albumin (urine fraction 2) or α_2 -euglobulin (urine fraction 4) was apparent.

XIE tests of the high molecular weight and 22 kd fractions against antidust (Fig. 4, A and B) and antiurine (not presented) revealed that the high-molecular-weight fraction consisted mainly of the specific dust component (Ag 1) and no urinary components, whereas the 22 kd fraction contained high amounts of the prealbumin (Ag 4), also Ag 3, and still with small amounts of Ag 1 (Fig. 4, B). When the 22 kd fraction was added to rat urine in XIE against antirat urine, only the urinary prealbumin peak was enlarged (Fig. 4, C). Compared to the 22 kd Ag 4 peak, the fraction of lower molecular weight (tubes 86 to 94) revealed a peak, antigenically identical to Ag 4, of slightly slower mobility, whereas in the fraction of higher molecular weight (tubes 66 to 76), it was slightly faster migrating (results not presented), indicative of a marked heterogeneity in this component with respect to molecular weight and pI (electrophoretic mobility).

The mean wheal diameter of skin prick tests in five rat-allergic patients with the original dust and the two main fractions (1 mg/ml) were (in decreasing potency) 22 kd fraction (Ag 4), 6.25; original F9 dust, 5.25; and high-molecular-weight fraction (Ag 1), 4.5. No

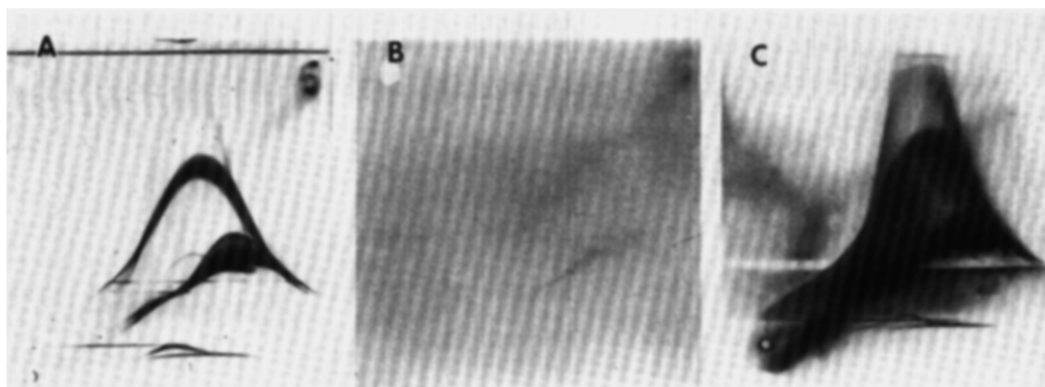


FIG. 5. XRIE tests of rat dust/antirat dust (compare with Fig. 1, A). A, Protein-stained plate. B, Autoradiograph with control sera. C, Autoradiograph with serum from rat-allergic individual.

reactions were obtained in six nonatopic subjects, two guinea pig-sensitive and two cat-sensitive subjects.

Rat dust extracts

XIE tests of extracts of other rat dusts obtained from various animal facilities demonstrated quite similar antigenic content to the F9 extract, although varying quantitatively, as observed from variation in their respective peak heights. For example, F8 (Fig. 4, D) obtained from a general rat-housing area, and F22 B (Fig. 4, E) obtained from a female rat room.

The XIE profile of the extract from the Staplex filter SI (Fig. 4, E) appeared to lack Ag 1, the high-molecular-weight specific dust component (as did only one other dust extract tested), but did contain significant amounts of the prealbumin component (Ag 4) that also exhibited a wide electrophoretic mobility. No serum components were detectable. When Staplex SI was added to a urine/antiurine XIE, the prealbumin peak was enlarged, leaving the reaction of partial identity to the α_2 -euglobulin (compare with Fig. 4, C), and in tandem XIE with saliva (not presented), a reaction of complete identity was observed.

XRIE

Fig. 5 illustrates the XRIE response to the rat dust/antirat dust. Fig. 5, A, illustrates the protein-stained template, with autoradiographs typical of those in Fig. 5, B, for control sera (three nonatopic and two atopic subjects, both reacting to guinea pig and house dust), and in Fig. 5, C, for sera from five rat-allergic individuals who all had high RASTs to rat dust and rat-urine extracts.² Compared to the strong uptake on all peaks, in particular Ags 1 and 4 for the sera from rat-allergic subjects, very little uptake of radioactivity was obtained with any of the control sera, not even

from the guinea pig-sensitive subjects, indicating a high degree of specificity for the test.

Quantitation of urinary prealbumin in rat dusts

Amounts of the prealbumin analogue component present in various dusts, as determined by rocket immunoelectrophoresis (Fig. 6, A and B), are presented in Table I. In the two instances in which male and female rat room dusts were sampled, the content of prealbumin was higher in that collected from the male rat rooms. It was also of interest that, because of inefficient maintenance of the electrostatic precipitators, similar levels were found on the inlet and outlet samples. The amount identified in the SI Staplex filter extract indicated a level of 37 ng of allergen per cubic meter of air.

DISCUSSION

The XIE profile of the rat dust/antidust proved to be quite different from profiles of either the rat urine/antiurine or rat saliva/antisaliva, and by testing the dust extract against the various heterologous antisera by a variety of quantitative immunoelectrophoretic techniques, it was apparent that, although some components or their "analogues" were present, many of the components of both urine and saliva (presumed to be the most likely sources of dust antigens) were absent (not detected) or present in only trace amounts.

The antigenic relationship of the component (Ag 4) in the rat dust to the major allergens identified in rat urine, i.e., prealbumin and its partially identical α_2 -euglobulin, (so defined from its electrophoretic mobility and insolubility in distilled water^{1, 5}) appears complex. The finding that this dust component is not

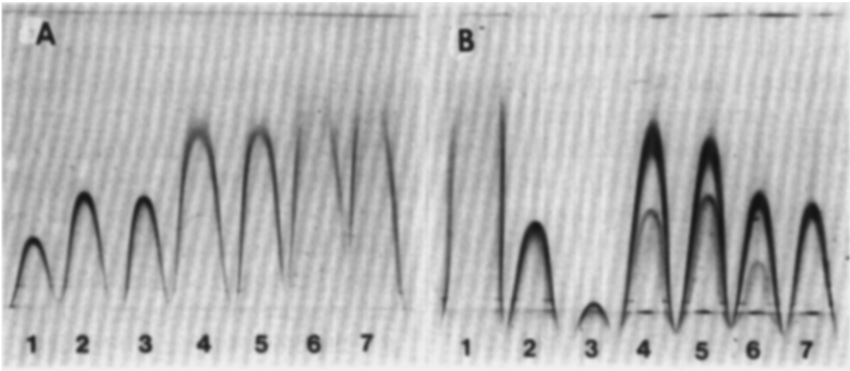


FIG. 6. Rocket immunoelectrophoresis of rat dusts with antiurinary prealbumin (fraction 3). **A**, Increasing concentrations of prealbumin standard (3, 5, and 7) alternating with those of the Staplex filter extract (1, 2, 4, and 6). **B**, Tenfold dilutions of prealbumin standard (1, 2, and 3) and dust extracts F22 A, inlet and outlet (4 and 5), F22 B, inlet and outlet (6 and 7) (prealbumin of dusts identified by reaction of identity with the standards).

completely identical to either would also argue that it does not originate from the rat urine, and this is further substantiated by the complete absence of the component with the electrophoretic mobility of the α_2 -euglobulin of male rat urine from dust collected from rooms housing male rats.

The presence of this component in dusts collected from female rat rooms would also indicate that it is not derived from α_2 -euglobulin, since α_2 -euglobulin, which is distinct from this protein, is not excreted in female rat urine.⁶

This component does, however, appear to share greater antigenic identity with urinary prealbumin, because, although it has a slightly slower electrophoretic mobility (as does its counterpart in saliva) when dust or the isolated fraction of rat dust was added to urine in a urine/antiurine XIE, the reaction of partial identity between the prealbumin and α_2 -euglobulin was maintained. Furthermore, when antiserum to the dust was incorporated in an IG of this profile, the effect observed on the prealbumin was greater than on the α_2 -euglobulin.

By gel filtration and isoelectric focussing (not presented) no component with molecular weights or pIs^{7, 8} of the α_2 -euglobulin were observed, whereas the fraction that eluted with a molecular weight very similar to that of the prealbumin of urine also demonstrated similar pI values. It was interesting to note, however, that the electrophoretic mobility (as assessed from the peak height) of a component demonstrating antigenic identity, but which eluted in fractions before the main dust prealbumin peak, migrated slightly faster, whereas in the fractions eluting after, it was slightly slower. Thus, in this component, a high degree of heterogeneity is indicated, both over molecular

TABLE I. Estimation of urinary prealbumin as percent of dry weight of dust in rat dust samples collected from various rat-housing areas

Prealbumin		
	Areas collected	% (dry weight)
Electrostatic precipitators		
F22 A	Male rat room	0.11*
B	Female rat room	0.075*
Extract fans		
F8	General rat room	0.25
F9	Male rat room	0.4
F10 A	Male rat room	0.25
B	Female rat room	0.16

*Amounts were the same for both inlet and outlet dust samples.

weight range and in electrophoretic mobility with these apparently being related to some extent, i.e., existence of several isomeric forms. However, when the latter eluting fractions, although components with pI values similar to the α_2 -euglobulin could not be identified, were added to the urine/antiurine, the α_2 -euglobulin peak appeared to increase. Therefore, possibly this dust prealbumin component is a form in which some of the antigenic sites unique to the urinary prealbumin (i.e., causing the reaction of partial identity) are missing, thus resulting in a more heterogeneous component with respect to molecular weight and pI.

It is of interest that, although the reaction of partial identity between the prealbumin and α_2 -euglobulin

with rabbit antiserum indicates a sharing of some but not all the antigenic IgG binding determinants of the prealbumin, in skin tests¹ and XRIE³ these components demonstrated the same reactivity, apparently indicating that the epitopes for human IgE binding were shared equally on both components. Cross-inhibition experiments have also indicated close similarity of human IgG binding by both components⁹; hence, the different epitopes revealed by the partial identity reaction with the rabbit antibodies may not be involved in the binding of either human IgG or IgE antibodies.

The α_2 -euglobulin originates in the liver and is concentrated in the kidneys,¹⁰ and although it is not presented in this article, when kidney or liver extracts were added to the urine/antiurine profile, it was the α_2 -euglobulin peak that was increased without any effect on the prealbumin (i.e., the reverse of adding saliva or dust extracts), and the reaction of partial identity between the components remained intact. This may indicate that, although these two components share a degree of antigenic identity, they in fact originate from different sources. That the dust prealbumin component shares antigenic identity with a similar component of saliva may indicate that its source is one of secretion.

From the marked difference in antigenic composition of the dust compared to those of urine and saliva, it is possible that urine and saliva are not the main source of the dust antigens as previously believed,^{3,4} even though fur is almost certain to become contaminated with both urine and saliva, and airborne dust particles could arise from such contaminated bedding. At that time it was also suggested that many of the antigens derived from urine and saliva may, after deposition on the fur, have undergone chemical modification or degradation that could account for the absence of some salivary/urinary components. However, at room temperature in the freeze-dried state, both the salivary and urine antigens appear quite stable, and also, *in vitro*, fresh saliva did not have any major proteolytic effect on the urine proteins or vice versa (personal observation). This finding would further suggest therefore that these are not the main sources of the dust antigens but, more likely, that they originate from skin secretions present on the fur.

In an earlier article,¹¹ the lack of allergenic activity in a rat "hair" extract was due to prior washing of the rats before removal of hair for extraction. Subsequent studies of rat-fur extracts (prepared without washing) have, however, demonstrated the potent allergenic nature of such extracts.^{3,12}

The presence on the stratum corneum and in the hair follicle of a major allergen, Ag 3 for the mouse,¹³ suggesting these as alternative sources of animal al-

lergens, could also indicate that the Ag 4 component of rat dust may originate from such sources, and studies are at present being carried out to investigate this possibility.

An alternative source for the dust components is further indicated by the presence in most of the dusts studied of a second major dust antigen, Ag 1, that could not be detected as present in either saliva or urine. Although the dust studied in most detail here had been accumulating on the filter for some months and therefore subject to possible degradation processes, the finding of this component in all but one of many samples collected from rat rooms in various animal-housing facilities leads us to believe that it is directly derived from the rats, but as yet its source of origin remains to be determined. Its absence from the Staplex filter sample was probably caused by the very small amount of dust that can be collected on such a filter, even after 4 to 5 hours of high-volume sampling. This component, which proved to be of quite high molecular weight (>200 kd), was demonstrated to be allergenic both by XRIE and in skin prick tests in rat-sensitive individuals and probably accounts for the inability of rat urine to completely inhibit rat dust RAST.¹²

The identification of the urinary prealbumin analogue, Ag 4, in the Staplex air filter sample suggests that determination of the levels of this component in air or dust samples will be a good indicator for the amounts of specific allergen present with regard to rat exposure, although α_2 -euglobulin, because of its close antigenic identity to prealbumin, has proved equally suitable.¹⁴ Amounts of Ag 4 in the various dusts demonstrated that the male rats appear to produce higher levels. This is in accordance with not only a higher urinary protein excretion^{1,15} but also, interestingly, a higher rate of secretion from their sebaceous glands^{16,17} than is observed in female rats.

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REFERENCES

1. Longbottom JL. Purification and characterization of allergens from the urines of mice and rats. In: Oehling A, Glazer I, Mathov E, Arbesman C, eds. *Advances in allergology and applied immunology*. Oxford: Pergamon Press, 1980:483-90.
2. Longbottom JL. Characterization of allergens from the urines of experimental animals. In: Kerr JW, Ganderton MA, eds. *Proceedings of the XI International Congress of Allergology and Clinical Immunology*. London and Basingstoke: Macmillan Press, 1983:525-9.
3. Walls AF, Longbottom JL. Quantitative immunoelectrophoretic analysis of rat allergen extracts. II. Fur, urine, and saliva

- studied by crossed radioimmuno-electrophoresis. *Allergy* 1983; 38:501-12.
4. Walls AF, Longbottom JL. Quantitative immunoelectrophoretic analysis of rat allergen extracts. I. Antigen characterization of fur, urine, saliva, and other rat-derived materials. *Allergy* 1983;38:419-31.
 5. Lane SE, Neuhaus OW. Further studies on the isolation and characterization of a sex-dependent protein from the urine of male rats. *Biochem Biophys Acta* 1972;257:461-70.
 6. Irwin JF, Lane SE, Neuhaus OW. Synergistic effect of glucocorticoids and androgens on the biosynthesis of a sex-dependent protein in the male rat. *Biochem Biophys Acta* 1971;252:328-34.
 7. Longbottom JL. Crossed radioimmunoisoelectric focusing as a method of identifying isoallergens: identification of isoallergens in rat urine extracts. *J Immunol Methods* 1984;68:297-304.
 8. Lane SE, Neuhaus OW. Multiple forms of $\alpha_2\mu$, a sex-dependent urinary protein of the adult male rat. *Biochem Biophys Acta* 1972;263:433-40.
 9. Platts Mills TAE, Longbottom JL, Edwards J, Cockcroft A, Wilkins S. Occupational asthma and rhinitis related to laboratory rats: serum IgG and IgE antibodies to the rat urinary allergen. *J ALLERGY CLIN IMMUNOL* 1987;79:505-15.
 10. Roy AK, Neuhaus OW. Proof of the hepatic synthesis of a sex dependent protein in the rat. *Biochem Biophys Acta* 1966;129:82-7.
 11. Newman Taylor AJ, Longbottom JL, Pepys J. Respiratory allergy to urine proteins of rats and mice. *Lancet* 1977;2: 847-8.
 12. Walls AW, Longbottom JL. Comparison of rat fur, urine, saliva, and other rat allergen extracts by skin testing, RAST, and RAST inhibition. *J ALLERGY CLIN IMMUNOL* 1985;75:242-51.
 13. Price JA, Longbottom JL. Allergy to mice. I. Identification of two major mouse allergens (Ag 1 and Ag 3) and investigation of their possible origin. *Clin Allergy* 1987;17:43-53.
 14. Platts Mills TAE, Heymann PW, Longbottom JL, Wilkins SR. Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. *J ALLERGY CLIN IMMUNOL* 1986;77:850-7.
 15. Alt JM, Hackborth H, Deerberg F, Stolte H. Proteinuria in rats in relation to age-dependent renal changes. *Lab Anim* 1980;14:95-101.
 16. Toh YC. Studies on the critical period of neonatal life for the differentiation of sebum secretion in rats. *Endokrinologie* 1982;79:7-12.
 17. Toh YC. The response of the sebaceous glands of female rats ovariectomized at birth with testosterone. *Endokrinologie* 1982;79:13-18.

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