

Laboratory animal allergy: the measurement of airborne urinary allergens and the effects of different environmental conditions

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Summary

Casella Simquad air samplers, with 0.5 μ M cut-off filters, were employed to sample the air in a laboratory animal house environment. The extracts obtained were assayed for laboratory animal urinary protein allergens using the inhibition radioallergosorbent test (RAST inhibition). The results showed that the collection and assay methods were of value and studies were extended to the influence of air change rates and humidity on airborne allergen levels. Reducing the air changes increased allergen levels, whilst increasing the humidity from 54% to 77% caused a significant reduction in allergen levels.

Interest in laboratory animal-induced allergy as an occupational health problem has increased markedly during recent years. There have been several major surveys of prevalence (Gross, 1980; Cockcroft, Edwards, McCarthy & Andersson, 1981; Davis & McArdle, 1981; Slovak & Hill, 1981) and several reports on the allergens involved have been published (Newman-Taylor, Longbottom & Pepys, 1977; Longbottom, 1979; Siraganian & Sandberg, 1979; Schumacher, 1980; Wahn, Peters & Siraganian, 1980). An important aspect of the problem, which has so far received little attention, is measurement of the levels of airborne allergen in animal house or laboratory environments. A recent publication (Twigg, Agarwal, Dahlberg & Yunginger, 1982) describes measurements of airborne allergens derived from mice. This paper extends those findings and describes a system for measuring airborne urinary allergens from mice, rats, guineapigs and rabbits and examines the effect of environmental changes on airborne allergen levels. Some of the results have been briefly reported by Dewdney (1981).

Materials and methods

Phosphate buffered saline (PBS) was obtained from Difco Laboratories (West Molesy, Surrey), and cellulose discs grade 541 paper, 0.6 cm in diameter, were obtained from Whatman Ltd

(Maidstone, Kent). All other chemicals were of reagent grade. Casella Simquad air samplers, catalogue number T13900/1 were obtained from Casella London Ltd (London N1 7ND) and used with standard filter heads (catalogue number T13070/1). Millipore glass fibre filters (number AP 2003500), 37 mm in diameter, were employed. Humidity measurements were carried out using a hygrometer purchased from Casella London Ltd. Human serum samples were obtained from clinically identified laboratory animal allergics. Air flow measurements were carried out using a meter, type TA 3000 manufactured by Airflow Developments Ltd (High Wycombe, England).

Air sampling

Initial studies (all species). Casella Simquad samplers were placed in various noted positions (see 'Results') in housing rooms primarily devoted to mice, rats, rabbits or guineapigs. Samplers were also placed in corridors outside housing rooms and one was placed at the dirty end of the cage washing area. Samplers were run for 5 h then the filters were removed, extracted and the extracts assayed.

Rat room studies: effect of varying air change rate and humidity. 1 rat housing room not the same as used for the initial studies (4.3 $\times$ 2.8 $\times$ 2.4 m) was stocked with 124 Sprague-Dawley rats in 4 racks of cages: 3 racks contained animals evenly distributed (36 animals per rack); in the 4th rack 16 animals were confined to one end of the rack, giving an empty and full end. The cages were cleaned out every morning and if the room conditions were to be altered from normal these changes were effected as early as possible so that the new conditions could stabilize. The air was then sampled overnight using 6 samplers placed in various positions (see 'Results'). These positions were maintained for the duration of the whole experiment.

Samples were collected (a) under normal environmental conditions; (b) with the air changes/h in the room reduced to about half the normal and temperature and humidity maintained as usual, and (c) with air changes/h and temperature

as normal but with relative humidity increased. The filter discs were extracted and then assayed for rat urinary allergens.

Elution of filters

After sampling, the filters were removed from the filter heads and folded into a 2 ml syringe. 1 ml of RAST buffer (PBS with 1% BSA, 1% Tween 20 and 0.1% sodium azide) was added to the syringe and the filter left to soak for 1 h. After this time it was squeezed dry using the syringe plunger and the eluent collected. This procedure was repeated with another 0.5 ml of RAST buffer. The volume of the eluent was measured and the samples were either assayed immediately or stored frozen until required.

RAST reagents and RAST inhibition assay

The urinary allergens and RAST discs were prepared as detailed elsewhere (Edwards *et al.*, 1983).

For the mouse, rat and rabbit urinary protein assays sera from suitable allergic individuals were initially diluted to 1/5 with RAST buffer; for the guineapig assay this initial dilution was 2/5. Standard solutions of the 4 urinary proteins were prepared at concentrations from 400 µg/ml to 400 pg/ml in log 10 steps in RAST buffer. All assays were carried out in triplicate in microtitre plates as follows. 25 µl aliquots of reagent serum were mixed with 25 µl of standard solution, or buffer or test solution, and after gentle mixing the plate was sealed with plastic film and incubated at 37° for 5 h. After this time the correct specificity RAST disc was added and the assay continued as described by Edwards *et al.* (1983).

A standard curve of cpm against concentration of standard allergen was plotted and test sample results were read from this curve.

Results

Room air flow system and environmental measurements

Using the Casella Simquad samplers with a flow rate through the filter of 2 l/min., we were able to study not only the levels of airborne allergen in the room but also their distribution. The air flow system in the housing rooms studied is illustrated in Fig. 1. Air enters through a central longitudinal slot in the ceiling and is extracted via 7 vertical wall-mounted droppers behind the cages. These droppers are perforated along their length (10 holes in 5 pairs) so that air is extracted at several vertical levels.

The airflow at each main extract duct in the ceiling (to which the droppers are attached) and at several positions along the inlet slot, was measured and these measurements were used to

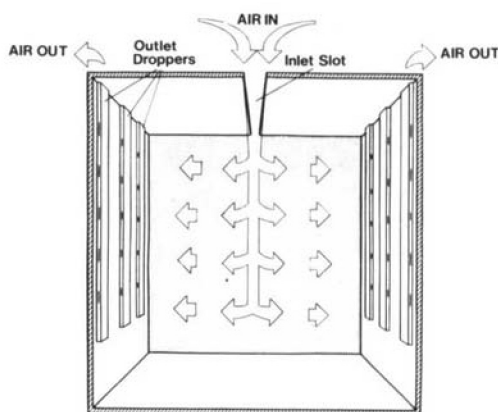


Fig. 1. Diagrammatic representation of the airflow pattern in an animal housing room

calculate the air changes/h in the room. Under normal conditions the room air was changed 14.7 times/h. Under reduced conditions (see below) this figure was 7.7 changes/h. For all these experiments the temperature of the room was maintained at $22 \pm 1^\circ\text{C}$ and normal relative humidity was $54 \pm 2\%$. For the rat room studies the humidity on one occasion was increased to a mean of 77%, range 71–82% during the sampling time.

Animal room sampling experiments

The results for the initial studies using all 4 species are presented in Table 1 (a to d).

For the mouse and rat rooms the allergen levels in the working areas (centre of room) were lower than those obtained from samplers behind the cages, suggesting that the air flow was concentrating allergen to the sides of the rooms. However the range of results obtained, especially for the rat room, may not justify this interpretation (see later discussion). Samplers placed in the corridors outside the mouse and rat housing rooms gave the lowest values either below the assay range or just measurable.

The results from samplers placed in the rabbit and guineapig housing rooms showed no concentration gradient peaking to the sides of the rooms but a sampler placed outside the rabbit room did give a relatively much lower result.

The sampler placed at the dirty end of the cage washing area provided some interesting results (Table 1d). They were all much lower than the respective housing room results. One suggestion was that this area has a higher relative humidity than normal in the animal house and therefore the influence of humidity was investigated (rat room study below).

Table 1. Results of initial airborne allergen sampling experiments

(a) Mouse room airborne allergen measurements

Sampler position	Allergen concentration (ng/m ³)
Behind cages	315.8
Behind cages	91.7
Working area	62.7
Working area	54.3
Outside room	9.6

(b) Rat room airborne allergen measurements

Sampler position	Allergen concentration (ng/m ³)
Behind cages	>44 000
Behind cages	683.3
Working area	336.2
Working area	274.0
Outside room	<44.0

(c) Rabbit room airborne allergen measurements

Sampler position	Allergen concentration (ng/m ³)
Working area	35 200
Behind cage	29 900
Working area	25 200
Behind cage	13 500
Working area	10 400
Working area	9 900
Outside room	1 700

(d) Guinea pig room airborne allergen measurements, with results from cage wash area sampler

Sampler position	Allergen concentration (ng/m ³)
Guinea pig room:	
Working area	50 600
Behind cages	42 400
Behind cages	22 300
'Dirty' end of cage wash area	14.5 mouse
	<48.0 rat
	604.8 guinea pig
	6000 rabbit

The results from Table 1 suggest that allergen levels in rabbit and guinea pig rooms are higher than for mouse and rat. This may be artefactual in that the relative purity of the 4 crude urinary extracts which are used as assay standards varies with respect to allergen (Lee, Edwards, Beeson & Dewdney, 1982). The rat and mouse crude allergens contain more allergen on a weight basis than the rabbit or guinea pig crude allergens and this leads to higher apparent results for the latter 2 species. Direct comparison between species results is not, therefore, justified by current techniques.

Environmental changes (rat housing room)

The results are presented in Table 2. The samples collected under normal conditions gave a similar picture to the rat room results in Table 1 with generally lower allergen levels being present in

Table 2. Influence of room conditions on allergen concentrations in a rat housing room

Sampler position	Room Condition Allergen concentration (ng/m ³)		
	Normal	Reduced air changes	Increased humidity
Behind cages	3 000	7 100	421
Behind cages	2 500	9 000	430
Behind cages	743	10 800	183
near extract duct			
Centre of room	582	4 200	281
Top of full rack	2 100	9 900	212
Top of empty half of a rack	116	1 400	40

the centre working area of the room. Of particular interest, however, was the 18-fold difference in allergen level between the sampler placed on the top of a full rack of animals and the sampler on the unpopulated half of a rack. These results emphasize the importance of using several samplers for air flow studies.

Under conditions of reduced air changes/h all results were increased by between 2 and 15-fold — range 1400 to 10 800 ng/m³ — however, with normal air changes/h resumed but with increased humidity, allergen levels fell to between 1/2 and 1/10 of normal, with no result being greater than 430 ng/m³.

Discussion

As part of a programme of work to investigate laboratory animal-induced allergy, we included studies on the measurement of airborne allergen levels in an animal house environment. Twiggs *et al.* (1982) have recently published some studies of this type for mouse allergens using a high volume air sampler and RAST inhibition for measurements. We also opted for measurement of the allergen using RAST inhibition, but employed small battery-powered samplers for air monitoring. These samplers draw air through a filter at a rate of only 2 l/min, and this low flow rate enabled us to employ several samplers in a room in an attempt to study the air flow pattern.

The initial results demonstrated that both the sampling procedure and assay method were satisfactory and the results for the mouse room were within the range quoted by Twiggs *et al.* (1982).

For the guinea pig and rabbit rooms, the results from the working area and behind the cages were similar, suggesting that the air extraction system was inadequate. This may be related to animal cage design. Certainly, the rabbit cages present a barrier to air movement in that they are designed with solid backs and sides. Further study is needed on this important point.

From the mouse and rat room results the initial impression was that allergen levels behind the cages were higher than those in the working area. This conclusion may not be justified, since in both cases only one sampler behind the cage has given a result much higher than the working area result. Also as can be seen from the results in Table 2, the proximity of the samplers to the animals does have a profound effect on the result obtained. The sampler on top of the full rack of animals gave a result close to those obtained from behind the cage samplers. The lessons to be learnt from this aspect of the work are that a large number of samplers would be required to monitor confidently air movements with respect to allergen concentrations, and single samples should not be relied on to assess a room's allergen level, since this will fail to identify high spots.

From the second rat room experiment (Table 2 results) two points emerge. The normal air flow in the room was not of itself causing allergen to become airborne by turbulence, since reducing the air flow substantially increased the allergen measurable. Secondly, increasing the humidity dramatically reduced allergen levels and could be used as an alternative to the expensive procedure of increasing air changes. However, the increased humidity may bring animal husbandry problems and also may not be occupationally acceptable for laboratory animal workers. We have no evidence on this matter but clearly any change

recommended would have to be fully assessed on all relevant parameters.

The wider issue is whether it would be economically viable to reduce animal house allergen levels to the point where an already sensitized individual would not be challenged. Individual sensitivity varies and this would make the setting of allergen threshold limit values extremely difficult, if not impossible. Even very sophisticated environmental control systems are upset by the presence of laboratory personnel. Finally, animals have to be handled extensively during experiments, and this in itself could lead to sudden high local allergen concentrations. It may, therefore, be more appropriate, in conjunction with reasonable measures to control animal house allergen levels, to consider local control by using personal protection or small laminar flow work stations. All these matters deserve further analysis. However, the conclusions from our study are that the combination of Casella Simquad air samplers and RAST inhibition provides a simple procedure for measuring allergen levels in animal rooms with enough sensitivity to study the effects of changing the room environment.

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Labortier-Allergie: Messung von aerogenen Urinallergenen und Einfluß verschiedener Umweltbedingungen**R. G. EDWARDS, M. F. BEESON & JANET M. DEWDNEY****Zusammenfassung**

Casella Simquad Luftsammler, mit 0.5 μ M Cut-off-Filtern, wurden zur Luftprobenentnahme in einem Versuchstierhaus verwendet. Die gewonnenen Extrakte wurden auf versuchstierspezifische Urin-Protein-Allergene unter Verwendung des Radioallergosorbent-Hemmungstest (RAST Inhibition) hin überprüft. Die Ergebnisse zeigten, daß die Methoden der Probenentnahme und Auswertung brauch-

bar waren. Die Untersuchungen wurden auf den Einfluß des Luftwechsels und der Luftfeuchte auf aerogene Allergenspiegel ausgedehnt. Eine Verminderung des Luftwechsels erhöhte die Allergenspiegel, während die Erhöhung der Luftfeuchtigkeit von 54 auf 77% eine signifikante Verminderung der Allergenspiegel erbrachte.

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