

Dust from carpeted and smooth floors.

V. Cat (*Fel d I*) and mite (*Der p I* and *Der f I*) allergen levels in school dust. Demonstration of the basophil histamine release induced by dust from classrooms

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Summary

Sixty dust samples from schools in Norway were analysed for major allergens from cat and mite after sampling with the regularly used vacuum cleaners for 5 days and with a new model vacuum cleaner for 10 days, respectively. The major feline allergen *Felis domesticus* allergen I (*Fel d I*) was detected in all the classrooms, with ranges from 12 to 16 840 ng/m² floor area. The mean *Fel d I* concentration was about 11 times higher per unit area carpeted floors as compared with smooth floors after the 10 days sampling period. Mite allergens *Dermatophagoides pteronyssinus* allergen I (*Der p I*) and *Dermatophagoides farinae* allergen I (*Der f I*) were detected in very low concentrations, with ranges from < 1 ng to 104 ng/m² floor area. These findings suggest that the school is a protective environment against mite infestation, while the prevalence of cat allergens in classrooms seems higher than previously assumed. Basophil histamine release was measured after provocation with 20 dust extracts from 10 different schools. In nine of the 10 schools examined, the basophil histamine release caused by challenge with carpet dust was higher than the corresponding release with smooth floor dust. The calculated floor areas in each school in which dust led to 15% histamine release were from 2 to 55 times larger for smooth floors compared with carpeted floors. These results emphasize previous findings regarding higher allergen concentrations in classrooms with carpeted floors.

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Introduction

Previous studies from our laboratory have illustrated that school dust may contain many different detectable allergens such as cat, dog, food, mite, mould and pollen [1,2]. Analyses of identical amounts of dust from carpeted and smooth floors showed higher contents of allergens of cat, dog, hen egg white, codfish, mould and timothy pollen in classrooms with carpeted floors [2]. We further investi-

gated the dust and allergen accumulation in classrooms by use of a new model vacuum cleaner [3]. The carpeted floors accumulated four to five times more dust and proteins per unit area compared with smooth floors. On the basis of the difference in dust content, significantly more allergens of pollen, cat, dog, food (egg white and fish), mite and mould were detected in the carpeted floors.

In the present investigation, the *Dermatophagoides pteronyssinus* allergen I (*Der p I*), *Dermatophagoides farinae* allergen I (*Der f I*) and *Felis domesticus* allergen I (*Fel d I*) contents per square metre floor area and per gram dust were measured in 60 classrooms. Furthermore, the histamine release from pre-sensitized basophils was estimated after provocation with 20 of the dust samples.

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Materials and methods

Preparation of the dust samples

In each school dust samples were collected from classrooms with carpeted and smooth floors by vacuuming the whole floor areas thoroughly daily after school hours. All samples were collected during the winter (December to February). Forty samples from 10 different schools were collected using the schools' regular vacuum cleaners for 5 days as previously described [2]. In a subsequent investigation, 20 samples from the 10 schools were collected using a new model vacuum cleaner for 10 schooldays [3]. The range of the vacuumed floor areas varied from 40–220 m². The dust was extracted in 50 mM NH₄HCO₃ and the dust extracts were filtered, centrifuged and refiltered as previously described [2,3]. The dust extracts underwent extensive dialysis (membrane cut off 3500 daltons) in 0.5 mM NH₄HCO₃ for 24 hr. The dialysed extracts were lyophilized and stored at –80°C until used in allergen analyses and basophil histamine release experiments. Lyophilized material per unit area in each classroom was calculated by multiplying the weight of lyophilized material obtained per gram of extracted dust with the total weight of vacuumed material per unit area.

Enzyme linked immunosorbent assay (ELISA) procedure

Reagents for the *Der p* I, *Der f* I and *Fel d* I sandwich ELISAs were obtained from Dr Martin D. Chapman, University of Virginia, Charlottesville, U.S.A. The reference cat dander preparation OBRR Cat E3 was obtained from Dr Christine Anderson, Office of Biologics and Research Resources, Department of Health and Human Services, Bethesda, Maryland, U.S.A. The assays were performed according to methods described previously [4–6]. In brief, microtitre plates were coated with anti-*Der p* I monoclonal antibody (MoAb) 5H8 C12 D9 [7], anti-*Der f* I MoAb 6A8 B10 D12 [8] or anti-*Fel d* I MoAb 6F9 A4 H1 [5]. The wells were blocked with 1% bovine serum albumin. Aliquots of 100 µl of solutions of lyophilized dust extracts (1 and 10 mg/ml for the mite ELISA; 0.1 and 1 mg/ml for the *Fel d* I ELISA) were added. Dilutions of reference *D. pteronyssinus* extract UVA 87/03 or *D. farinae* extract UVA 87/02 [4] from 1 to 250 ng/ml were used for the control curves. For the *Fel d* I ELISA, control curves were established using dilutions of the reference cat dander from 26 to 0.5 mU/ml. The wells were further incubated with biotinylated anti-*Der* I MoAb 4C1 B8 3F8 [9] or anti-*Fel d* I MoAb 3E4 C4-C10 [5]. After incubation with streptavidin-peroxidase, the plates were developed and read at 414 nm using a Titertek Multiskan MCC plate reader (Flow Laboratories, Rickmansworth, Hertfordshire, U.K.). The concentration of *Fel d* I was calculated

assuming 1 U *Fel d* I = 4 µg protein [5]. The allergen contents in each classroom were calculated on the basis of weights of lyophilized material per unit area and per gram of dust, respectively.

Leucocyte separation, lactic acid stripping and passive sensitization

Blood was obtained after an informed consent from volunteers who were non-atopics. Thirty millilitres of blood were mixed with 7.5 ml 6% Dextran 70 in physiological saline, 225 mg dextrose and 3 ml 0.1 M ethylene diamine tetra-acetic acid, disodium salt, pH 7.6. The mixture was allowed to sediment at room temperature for 90 min before the leucocyte-containing plasma was removed and centrifuged (220 g, 8 min, 4°C). The leucocytes were washed twice with PIPES-albumin (PA) buffer (piperazine-*N,N*-bis-2-ethane sulphonic acid 25 mM, NaCl 110 mM, KCl 5 mM, NaOH 40 mM and human serum albumin 0.03%, adjusted to pH 7.4), once with PAG (PA with 0.1% D-glucose) and once with physiological saline before resuspending the cells in lactic acid solution (lactic acid 10 mM, NaCl 140 mM, and KCl 5 mM, adjusted to pH 3.9) for 3.5 min [10]. The cells were resuspended in PA, and passive sensitization was accomplished by the method of Levy and Osler [11], using sera from multi allergic patients with IgE against cat, dog, mite and dust (RAST classes 2–3). The cells were washed twice in PA and resuspended in D₂O-PACM (PA with CaCl₂ 1 mM, MgCl₂ 1 mM and 50% D₂O) for histamine release studies.

Histamine release

Freshly prepared and sterile filtered solutions of lyophilized dust extracts in concentrations from 4 to 1200 µg/ml in D₂O-PACM were used for histamine release (HR) studies. Amounts of 200 µl of the lyophilized dust extract dilutions were added to 200 µl aliquots of leucocyte suspension and incubated for 60 min at 37°C. In addition, leucocytes were incubated with buffer alone to correct for the spontaneous HR. The leucocyte number was chosen so that each tube contained 20–40 ng of histamine (2–3 × 10⁴ basophils). A control set of unsensitized basophils was similarly challenged by the highest concentrations of the dust extracts. Total cellular histamine was obtained by freezing and thawing five times in dry ice-ethanol mixture. The tubes were centrifuged (600 g, 10 min), and the supernatants assessed for histamine content by a radioimmunoassay technique (Pharmacia Histamine Radiimmunoassay, Pharmacia AB, Uppsala, Sweden) [12]. An antigen blank was included to correct for a possible content of histamine in the dust extracts. The

mean of duplicate determinations was calculated, and HR was expressed as percentage of total cellular histamine according to the formula

$$\% \text{ HR} = \frac{(\text{stimulated HR} - \text{spontaneous HR} - \text{control HR})}{\text{total cellular HR} - \text{spontaneous HR}} \times 100$$

The amount of dust in milligrams and the corresponding floor area in square centimetres required to release 15% histamine were calculated based on the individual histamine release curves.

Statistics

The Wilcoxon matched-pairs test was used to compare the different parameters in dust samples from classrooms with carpeted and smooth floors. The limit of significance chosen was 0.05.

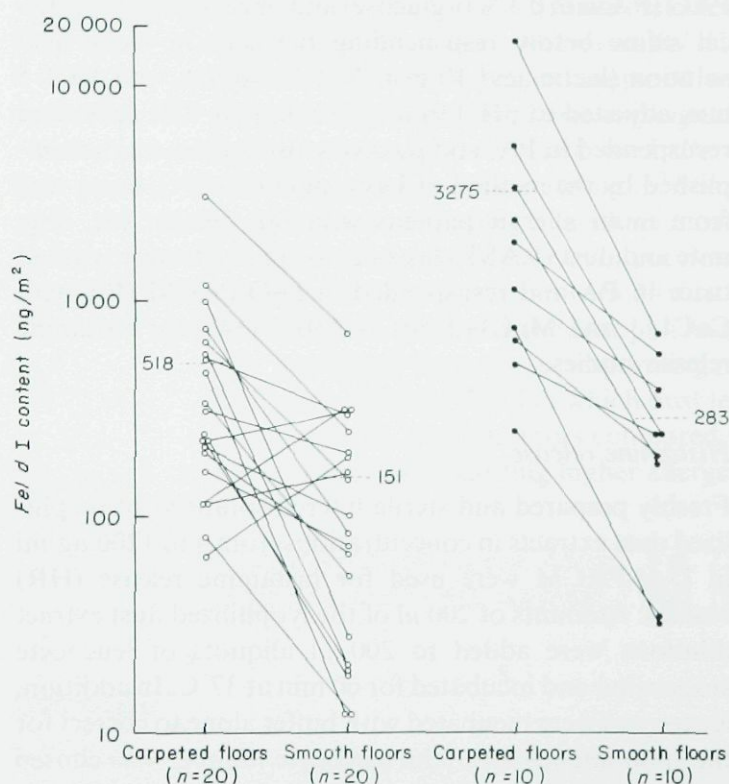


Fig. 1. *Fel d I* content (ng/m² floor area) as determined for 40 classrooms vacuumed daily with the schools' own vacuum cleaners for 5 schooldays (○) and 20 classrooms vacuumed with a new model vacuum cleaner for 10 schooldays (●). The lines designate results from paired sampling of classrooms with carpeted and smooth floors in the same school. Differences between arithmetic mean (dashed line) of carpeted and smooth floors were statistically significant for both the five days sampling period (means, 518 versus 151 ng/m²; $P=0.0008$) and 10 days sampling period (means, 3275 versus 283 ng/m²; $P=0.001$).

Results

Vacuumed material weights in the classrooms have been reported previously and varied widely from 0.2–14.1 g/m² [2,3]. The new model vacuum cleaner recovered more dust from the carpet 'reservoir' than the schools' regularly used vacuum cleaners [3]. The content of *Fel d I* as estimated in nanograms per square metre floor area varied from 12 to 16 840, as illustrated in Fig. 1. Sampling of dust using the regular vacuum cleaners for 5 days yielded a mean *Fel d I* content more than three times higher in classrooms with carpeted floors compared to classrooms with smooth floors (518 ng/m² versus 151 ng/m²; $P=0.0008$). Furthermore, dust sampling with the new model vacuum cleaner for 10 days gave a mean *Fel d I* content in classrooms with carpeted and smooth floors of 3275 ng/m² and 283 ng/m², respectively ($P=0.001$). Hence, a difference of more than 11 times in the *Fel d I* concentration in carpeted versus smooth floors was found. As expected, the amount of *Fel d I* on the smooth floors was about twice as high after the 10 day sampling period compared to the 5 days sampling period. On the contrary, the mean *Fel d I* content on the carpeted floors was about six times higher after the 10 day sampling period using the new vacuum cleaner (3275 ng/m² contra 518 ng/m²).

The content of *Fel d I* in nanograms per gram of coarse dust is shown in Fig. 2. Mean values for carpeted and smooth floors were respectively 262 ng/g and 107 ng/g after the 5 days sampling period. By using the new model

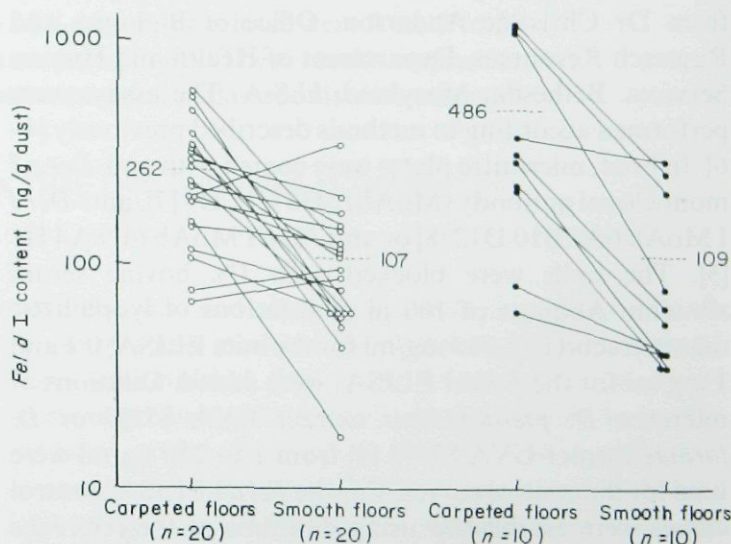


Fig. 2. *Fel d I* content (ng/g of dust) as determined for classrooms with carpeted and smooth floors. The sampling periods and symbols are described in Fig. 1. Differences between arithmetic mean (dashed line) of carpeted and smooth floors were statistically significant for both the five days sampling period (means, 262 versus 107 ng/g; $P=0.0003$) and 10 day sampling period (means, 486 versus 109 ng/g; $P=0.001$).

Table 1. *Der p I* and *Der f I* contents in ng/m² floor area and ng/g of unsieved dust in classrooms with carpeted (CF) and smooth (SF) floors after the two sampling periods of 5 and 10 days, respectively. Arithmetic means and ranges are given

	5 days		10 days	
	CF (n=20)	SF (n=20)	CF (n=10)	SF (n=10)
<i>Der p I</i>				
ng/m ²	< 13 (< 1-104)	< 3 (< 1-21)	< 15 (< 1-33)	< 8 (< 1-34)
ng/g	< 5 (< 1-38)	< 2 (< 1-8)	< 3 (< 1-5)	< 3 (< 1-18)
<i>Der f I</i>				
ng/m ²	< 7 (< 1-< 38)	< 5 (< 1-< 36)	< 9 (< 1-< 17)	< 6 (< 1-< 10)
ng/g	< 3	< 3	< 2	< 2

vacuum cleaner, the mean *Fel d I* content of carpet dust increased to 486 ng/g, while the mean content in dust from smooth floors remained constant. The differences in *Fel d I* content per gram dust between carpeted and smooth floors were significant ($P \leq 0.001$).

The *Der p I* content in the dust samples was low, 52/60 classrooms containing less than 20 ng *Der p I*/m² and 59/60 less than 20 ng *Der p I*/g of dust. The *Der f I* content was almost unmeasurable, 53/60 samples containing less than 10 ng *Der f I*/m² and all samples less than 10 ng *Der f I*/g of

dust. The *Der p I* and *Der f I* contents per square metre and per gram of dust are shown in Table 1. Carpeted floors contained significantly more of *Der p I* per square metre than smooth floors ($P < 0.02$). Vacuuming with the new model vacuum cleaner did not increase the *Der p I* and *Der f I* contents per square metre of floor area or per gram of dust. The estimated total concentrations of *Fel d I*, *Der p I* and *Der f I* in micrograms in floor dust are given in Table 2.

The basophil HR induced by dust from 10 different schools is shown in Fig. 3. Basophils from two different non-atopic donors (TD and JH) were used in this investigation. Pre-sensitized basophils from donor JH

Table 2. Mean total concentrations (μg) of *Fel d I*, *Der p I* and *Der f I* in floor dust in classrooms with carpeted (CF) and smooth (SF) floors after the two sampling periods of 5 and 10 days, respectively. Arithmetic means, standard deviations and ranges are given where appropriate

	5 days		10 days	
	CF (n=20)	SF (n=20)	CF (n=10)	SF (n=10)
<i>Fel d I</i>	31.7 $\pm$ 26.2 (6.7-121.1)	7.5 $\pm$ 5.9 (1.1-19.1)	180.2 $\pm$ 185.9 (22.3-522.1)	17.4 $\pm$ 18.5 (2.2-66.1)
<i>Der p I</i>	< 0.7 (0.06-4.2)	< 0.2 (0.02-0.6)	< 1 (< 0.2-2.3)	< 0.7 (< 0.07-4.1)
<i>Der f I</i>	< 0.5	< 0.2	< 0.6	< 0.4

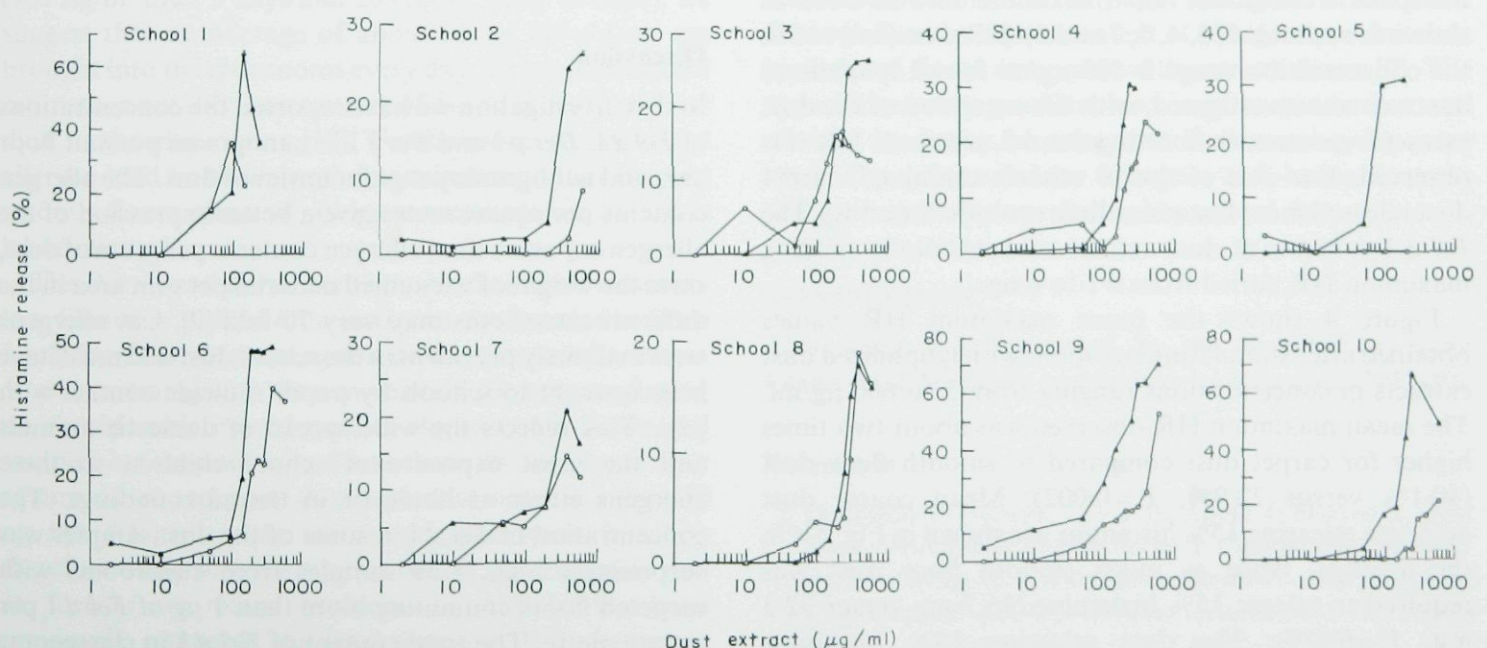


Fig. 3. Basophil histamine release (%) induced by solutions of lyophilized dust extracts from carpeted ($\blacktriangle$) and smooth ($\circ$) floors in 10 schools.

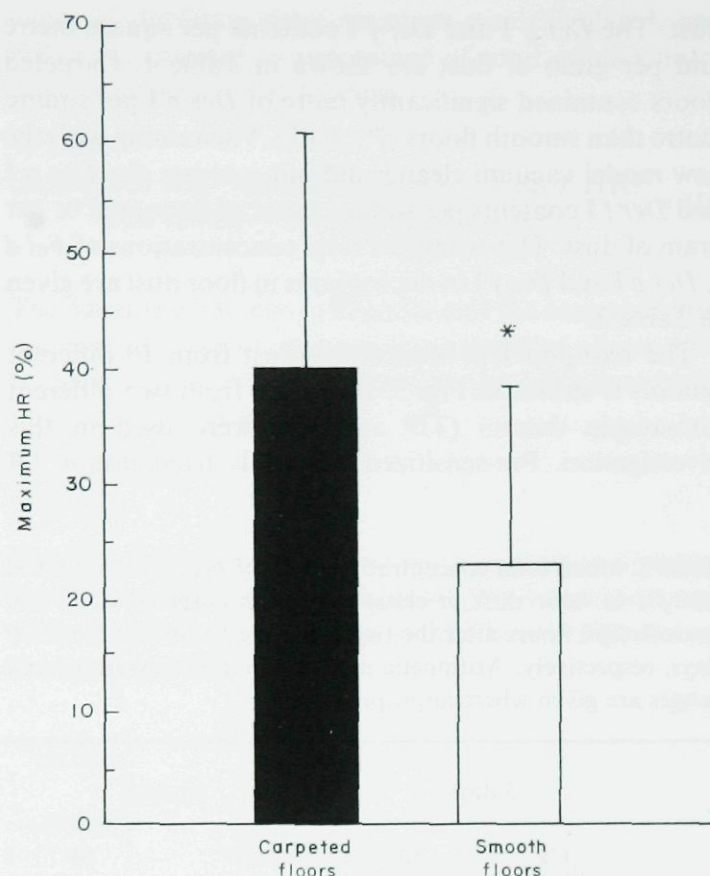


Fig. 4. Maximum histamine release (HR), expressed as mean percentage $\pm$ s.d., observed after stimulating basophils with dust extracts from classrooms with carpeted ($n=10$) and smooth ($n=10$) floors. * $P=0.002$.

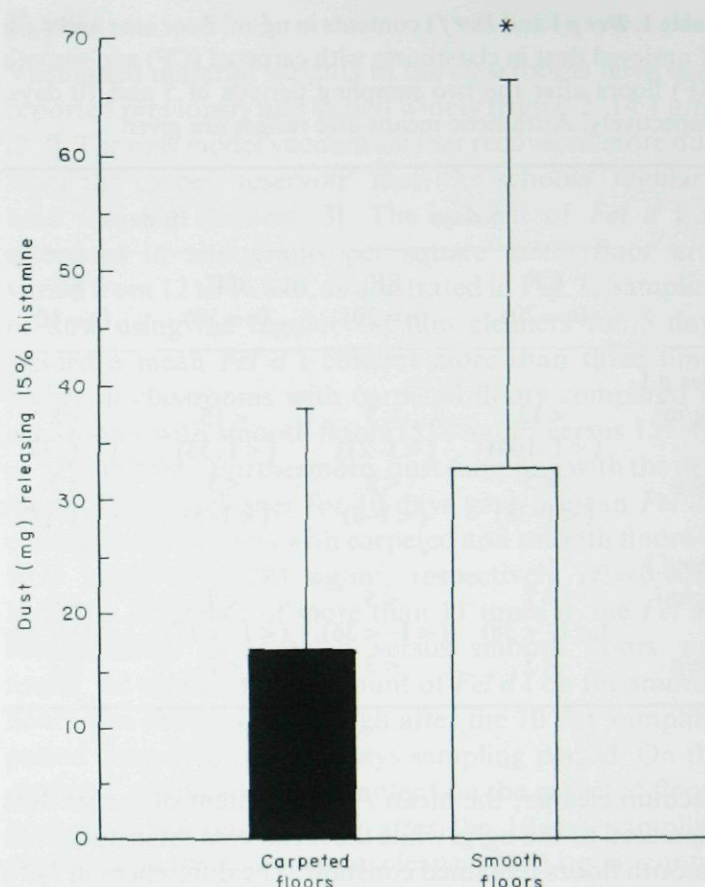


Fig. 5. Mean coarse dust amount ($\text{mg} \pm \text{s.d.}$) from classrooms with carpeted ($n=10$) and smooth ($n=10$) floors required to release 15% histamine. * $P=0.010$.

released more histamine (maximum HR 50–80% as shown for schools 1, 6, 9 and 10) than the corresponding basophils from donor TD (maximum HR 20–30% as shown for schools 2, 3, 4, 5, 7 and 8). HR was observed in the concentration range 2–600 $\mu\text{g}/\text{ml}$ for all lyophilized dust extracts investigated, with the exception of the dust extract from smooth floor in school 5, where no HR was observed. For nine of the 10 schools examined, carpet dust released more histamine than smooth floor dust. The *Fel d I* contents at dust extract concentrations yielding maximum HR varied from 0.1 to 6 ng.

Figure 4 shows the mean maximum HR values obtained after stimulating basophils with lyophilized dust extracts in concentrations ranging from 2 to 600 $\mu\text{g}/\text{ml}$. The mean maximum HR observed was about two times higher for carpet dust compared to smooth floor dust (40.1% versus 22.9%, $P=0.002$). Mean coarse dust amounts releasing 15% histamine are shown in Fig. 5. On the average, twice as much smooth floor dust was required to release 15% histamine (16.7 mg versus 32.3 mg, $P=0.010$). The dust releasing 15% histamine stemmed from smooth floor areas on the average eight (range 2–55) times larger than carpeted floor areas, as

shown in Fig. 6. The results in Figs 4, 5 and 6 showed a large interschool variability, and the standard deviations were hence large.

Discussion

In this investigation we have reported the concentrations of *Fel d I*, *Der p I* and *Der f I* in nanograms per unit floor area and nanograms per gram unsieved dust. The allergen contents per square metre give a better expression of the allergen exposure than allergen contents per gram of dust, since the weight of vacuumed material per unit area in the different classrooms may vary 70-fold [2]. Cat allergens were extremely prevalent in the school dust and must have been brought to schools by pupils' outside contact with pets. This reflects the wide spread of domestic animals and the great exposure of school children to these allergens either at home or in the surroundings. The concentration of *Fel d I* in some of the dust samples was surprisingly high, 8/30 samples from classrooms with carpeted floors containing more than 1 μg of *Fel d I* per square metre. The total content of *Fel d I* in classrooms with carpeted floors was on an average 10 times higher than for classrooms with smooth floors after sampling

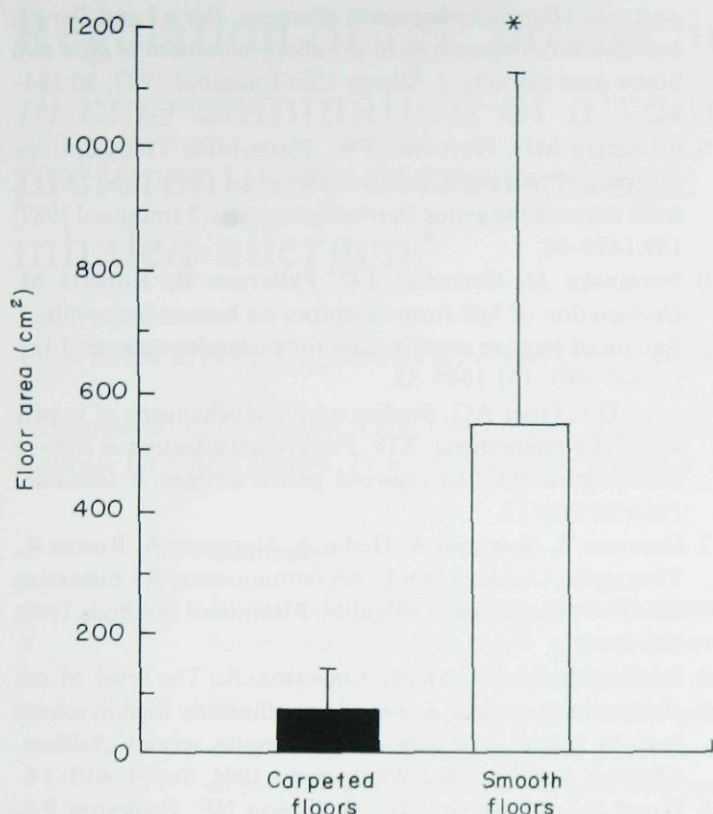


Fig. 6. Mean floor area ($\text{cm}^2 \pm \text{s.d.}$) of classrooms with carpeted ($n=10$) and smooth ($n=10$) floors the dust content of which released 15% histamine. * $P=0.001$.

with the new model vacuum cleaner, suggesting removal of cat allergens from the carpets' dust reservoir. Since the mean amounts of *Fel d I* in dust from smooth floors were rather constant after the 5 and 10 days sampling periods (151 ng/m^2 after 5 days and 283 ng/m^2 after 10 days), we suggest that an average of about $30 \text{ ng } Fel d I/\text{m}^2$ are brought into the classrooms every day. This is a minimum estimate, since there are sources of *Fel d I* other than floor dust in the classrooms [13]. Furthermore, the constant allergen load on the smooth floors indicated that keeping smooth floors clean of allergen accumulation is not a laborious task.

The *Der p I* and *Der f I* contents in school dust were very low. Contrary to *Fel d I*, no increase was observed in *Der p I* and *Der f I* contents per gram dust or per unit floor area after vacuuming with the new vacuum cleaner, suggesting no considerable mite depots in the carpeted floors. All samples but one contained less than $100 \text{ ng } Der p I$ per square metre and gram dust and all samples less than $40 \text{ ng } Der f I$ per square metre and gram dust. It seems therefore likely that the mite species *D. pteronyssinus* and *D. farinae* are minor problems in schools. We have previously estimated the total potency of *D. farinae* in school dust by radioallergosorbent test (RAST) inhibition, detecting allergenic activity [1–3]. Contrary to the

ELISA, where the MoAb in the coating is *Der f I* specific, the RAST inhibition measures total allergenic activity (detects the whole range of allergens derived from *D. farinae*) and in addition the human IgE antibodies crossreact with *D. pteronyssinus* and possibly with other mite species.

In our study, no dust sieving process was used, and the vacuumed material extracted contained large amounts of non-antigenic material. The mean protein content (w/w) of the coarse dust extracted was less than 1% and the mean weight of dialysed material was 1–2% of the dust weight extracted [2,3]. The levels of *Der f I*, *Der p I* and *Fel d I* reported per gram of dust in our investigation will hence be lower than in other investigations using sieved dust. Wood *et al.* [14] reported that the content of *Fel d I* in the extract of unsieved dust was less than that of the fine dust extract, with unsieved to sieved ratios ranging from 0.05 to 0.91 (median 0.29).

Basophil histamine release was used to assess the allergenic activities of school dust preparations. We established a test system using pre-sensitized basophils, stimulated these with the dust extracts and could then directly compare the HR caused by dust from classrooms with carpeted and smooth floors in the same school. The 15% HR values were chosen to be able to compare parameters for most dust extracts tested. Since sera from patients with multispecific IgE against several allergens (cat, mite, pollen) were used in the sensitization procedure, any correlation between the *Fel d I* content in the dust samples and the basophil HR could not be found. The results from the allergen detection and HR experiments further confirm our previous findings regarding considerable amounts of depot allergens in carpeted floors [1,3] and a higher content of allergens per unit dust in carpeted floors compared with smooth floors [2]. The apparent advantage of linoleum over carpet in dust and allergen load may be important in decreasing exposure level of pupils to indoor allergens and hence decrease allergic manifestations during the school day.

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