

Dust from carpeted and smooth floors

II Antigenic and allergenic content of dust vacuumed from carpeted and smooth floors in schools under routine cleaning schedules

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Dust samples were collected twice from smooth and carpeted floors in 10 Norwegian schools. The content of antigens and allergens of alder (*Alnus incana*), birch (*Betula verrucosa*), timothy (*Phleum pratense*), cat and dog dander, house dust mite (*Dermatophagoides farinae*), mould (*Cladosporium herbarum*), hen egg white and codfish (*DIII*) were investigated by crossed immunoelectrophoresis (CIE), crossed radio immunoelectrophoresis (CRIE), radio allergosorbent test (RAST) inhibition and quantitative precipitation inhibition analysis by laser nephelometry. Antigens and allergens of cat and dog dander and hen egg white were most prevalent in the dust samples investigated. With the exception of hen egg white and codfish allergens, no statistically significant differences in mean allergen content were shown in identical quantities of freeze-dried dust extracts from carpeted and smooth floors. RAST-inhibition analyses of identical amounts of dust from either floors showed higher content of allergens of cat, dog, hen egg white, codfish, mould and timothy pollen in classrooms with carpets.

Key words: allergens; carpeted floors; dust; schoolchildren.

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The aerobiology in schools and public institutions has been partially investigated in several studies on indoor climate (1, 5, 11). Carpets in schools and other public buildings have been suspected to be a risk for patients with asthma and allergy (10, 15). Badly cleaned wall-to-wall carpets can lead to air pollution by dust particles or microorganisms causing various indoor-climate complaints (10-15). We have recently presented some qualitative and quantitative studies on the antigenic and allergenic proteins in dust collected from Norwegian schools (6). In contrast to reports from other laboratories these studies were performed under normal daily conditions. There were neither complaints of indoor climate nor water-damaged carpets in the schools selected, and the cleaning procedures were performed according to routines recommended by the Norwegian health authorities. No relation-

ship could be demonstrated between floor type and allergen concentration by analyses of 2 mg lyophilised dust extracts from carpeted and smooth floors.

In the present investigation the antigens and allergens of pollen, mould, house dust mite, animal dander and food were qualitatively and quantitatively analysed in dust samples collected from smooth and carpeted floors in schools. The aim of this study was to investigate the eventual differences in allergen content in these floors under regular conditions, when cleaning procedures were satisfactorily performed and no complaints about indoor climate were reported.

Smooth floors are usually washed and not vacuumed. Therefore, to simulate the actual daily practice the smooth floors were washed after collecting the dust samples. The dust ex-

tracts were dialysed and the retained proteins with molecular weight over 8,000 daltons were analysed.

MATERIAL AND METHODS

Sample collection

Dust material was collected twice, in January and December 1987, in 10 schools in east and west Norway. The 40 classrooms included in this study were furnished with either carpeting or smooth floor coverings. The dust was collected by vacuum cleaners equipped with filters and disposable bags. In each school, samples from two carpeted and two non-carpeted classrooms were collected by daily vacuum cleaning of the whole floor at the end of 5 schooldays. The same vacuum cleaner and a fresh disposable bag were used for each room included in the study. The smooth floor coverings were washed every day after vacuum cleaning as in routine cleaning schedules. Dust samples were sealed in plastic bags and mailed to the laboratory where they were stored at +4°C until extracted. A questionnaire was filled in with pertinent information: floor area in m², cleaning routines, frequency of vacuum cleaning, procedure and date for the last carpet cleaning, age of the carpet, room temperature, number of windows that could be opened, room function, topographic location of the school and technical data of the vacuum cleaner. The dust samples from carpeted floors (CF) and smooth floor coverings (SF) were numerically designated CF001–CF020 and SF001–SF020.

Extraction and lyophilisation

The dust extracts were prepared by suspension of 10 g dust in 50 mM NH₄HCO₃ (pH 8.0) to a total volume of 500 ml. The mixtures were gently shaken for 3 h at 4°C, filtered, centrifuged (6000 rpm for 45 min) and dialysed (cut-off 8000) for 24 h. The extracts were then filtered (0.45 µm), lyophilised and stored at –20°C until used. The antigenic proteins were

preliminarily estimated by the weight of freeze-dried preparation and expressed in mg lyophilised extract per g dust (10).

Allergens

Lyophilised extracts of *D. farinae* (4) and *C. herbarum* were kindly provided by Siri Dale, Nycomed, Oslo, Norway. Lyophilised extracts of timothy pollen, cat and dog dander were supplied by Allergologisk Laboratorium København (ALK), Copenhagen, Denmark. Crude hen egg white, partially purified codfish allergen (DIII) extract and crude birch and alder pollen extracts were prepared as described previously (7, 8, 9, 17).

Rabbit antisera

Rabbit antibodies against alder, birch and timothy pollen, cat and dog dander, house dust mite, mould, codfish and hen egg white extracts were produced as described previously (6, 12). Rabbit antiserum against human albumin was supplied by Norske Hoechst, Oslo, Norway.

Human sera

Sera from patients with Type I allergy to the selected allergens were collected from volunteer outpatients. Sera from minimum six patients with RAST classes 2–4 against the current allergen were pooled and stored at –20°C until used. Monospecific patient sera for cat, dog, alder and birch were difficult to obtain. RAST classes 2–4 for both cat and dog were allowed in some of the patient sera used. Similarly, RAST classes 2–4 for both alder and birch were allowed in the patient sera.

Protein analysis

The protein concentrations were estimated in aliquots of 5 mg/ml of the lyophilised dust extracts by the Lowry method (14), using bovine

Table 1

Mean values of weights of vacuumed material per m², weights of lyophilised extract recovered/g dust, protein concentrations in mg/100 mg lyophilised extract and protein concentrations in mg related to weight of dust and area of the classrooms. Arithmetic mean, standard deviation and range are given. The Wilcoxon matched-pairs test was used to compare the different parameters in dust from 20 carpeted and 20 smooth floors. The level of significance was 0.01.

	Carpeted floors (n = 20)	Smooth floors (n = 20)	P values
Vacuumed material, g/m ²	2.4±3.1 (0.2-13.1)	2.3±3.7 (0.2-14.1)	0.18
Lyophilised extract, mg/g dust	15.4±6.5 (6.7-29.1)	11.6±7.3 (3.5-31.7)	0.006
Protein, mg/100 mg lyophilised extract	26.1±8.9 (11.8-42.9)	22.2±8.2 (5.0-34.5)	0.008
Protein, mg/g dust	4.0±1.9 (1.5-8.1)	2.7±1.7 (0.8-6.9)	0.0001
Protein, mg/m ²	8.4±12.5 (1.1-59)	6.7±13.8 (0.2-59)	0.03

serum albumin as the standard reagent. The absorbance was read at 750 nm, and the protein estimates were calculated from standard calibration curves.

Immunoelectrophoretic techniques

Crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE) were performed as earlier described (18). Briefly, aliquots of 12.5 µl of solutions (20 mg/ml) of lyophilised dust extracts were applied in 3 mm diameter wells for the first dimension electrophoresis, using runs with 10 V/cm for 45 min. The second dimension gels in CIE contained respectively 8 µl/cm² antiserum against *D. farinae*, 10 µl/cm² antiserum against cat dander, 12 µl/cm² antiserum against crude hen egg white, dog dander, codfish (*DIII*) or crude timothy pollen, 24 µl/cm² antiserum against crude

Table 2

Number of samples from 20 carpeted and 20 smooth floors containing antigens (Ag) and allergens (Al) of alder, birch, timothy, cat, dog, mite, hen egg white, codfish and human serum albumin (HSA) as assessed by CIE and CRIE.

	Carpeted floors (n = 20)		Smooth floors (n = 20)	
	Ag	Al	Ag	Al
Alder	0	1	0	0
Birch	0	1	0	0
Timothy	0	0	0	0
Cat	20	20	20	20
Dog	20	20	20	20
Mite	0	0	1	0
Egg white	20	20	20	20
Codfish	0	1	0	0
HSA	20	ND	20	ND

Ag = antigen; Al = allergen; ND = not done.

alder or birch pollen and 6 µl/cm² antiserum against human serum albumin. Autoradiography was performed using dried plates on X-ray film (Kodak X-Omat AR) in a light-proof chamber. The films were exposed at -80°C for 2 days to 1 week and developed in a Kodak X-Omat RP automatic processor for 90 s at 33°C (13). As a control CIE plates were incubated with physiological saline to detect eventual unspecific binding of ¹²⁵I-anti-IgE to the immunoprecipitates.

Preparation of allergen discs

Allergen discs of egg white, alder, birch and codfish were made according to a procedure described by Yman et al. (19). Following cyanogenbromide activation (3), the discs were coated with the different allergen solutions at a concentration of 1 mg/ml. For hen egg white a 10% solution of the extract was used for coating. These discs were compared with the allergosorbent discs manufactured by Pharmacia using different patient sera by direct RAST. The correlation coefficient varied from 0.97-0.99. Other paper discs (*C. herbarum*, cat and dog dander, timothy and *D. farinae*) were purchased from Pharmacia AB, Uppsala, Sweden.

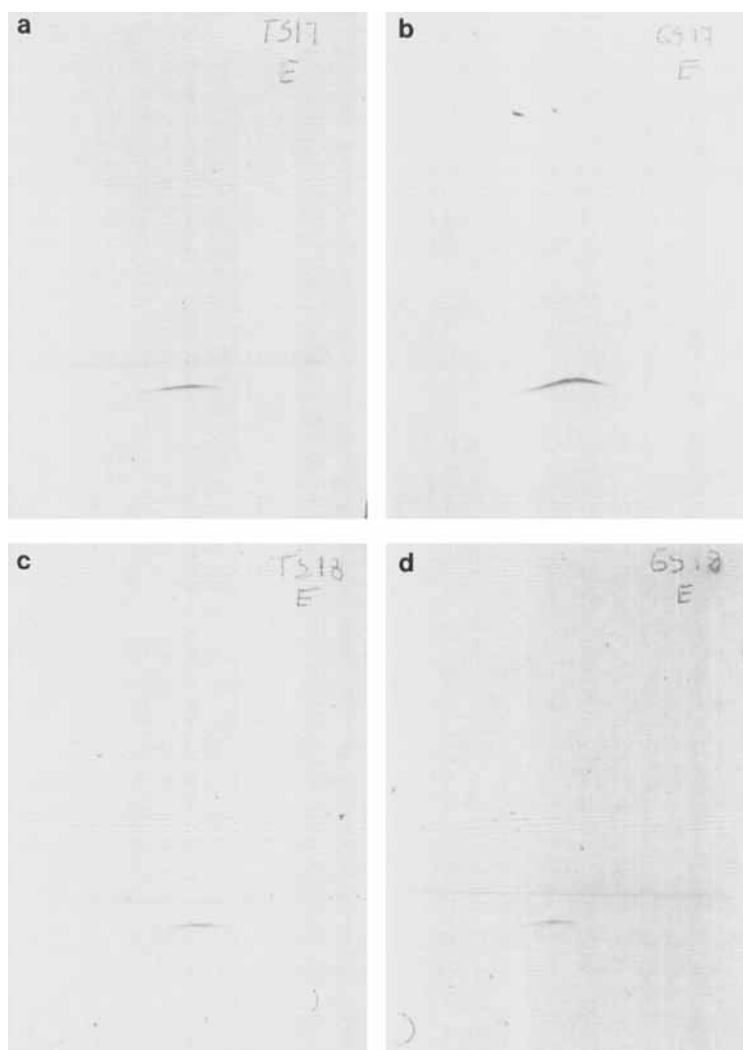


Fig. 1. CIE of 250 µg lyophilised dust extracts CF017 (a), SF017 (b), CF018 (c) and SF018 (d). The first dimension electrophoresis was performed towards the anode at 10 V/cm for 45 min; the second dimension electrophoresis at 2 V/cm for 20 h using 12 µ/cm² rabbit antiserum against crude hen egg white in the anodic antibody-containing gel.

RAST inhibition tests

The allergenic activity of the different dust extracts was tested *in vitro* by RAST inhibition titration studies (6, 19). The 50% inhibition values were not calculated due to the low concentrations and the great variation in contents of alder, birch and timothy pollen, mite and

hen egg white in the dust extracts (6). Briefly, an aliquot of 100 µl of a 20 mg/ml solution of lyophilised dust extract was added to 50 µl of patient serum pool and incubated for 1 h at room temperature. An allergen disc coated with the appropriate allergen was added to the mixture and incubated for 3 h. The disc was further incubated overnight with 50 µl ¹²⁵I-anti-IgE (Phadebas RAST isotope, Pharmacia AB, Uppsala, Sweden). The tests were performed in

duplicate. The value of per cent inhibition was calculated using the formula:

$$\frac{(\text{cpm of non-inhibited serum} - \text{cpm of inhibited serum}) \times 100}{\text{cpm of non-inhibited serum}}$$

In addition, RAST inhibition tests were performed in solutions of lyophilised dust extracts corresponding to 100 mg dust. The allergenic activities were confirmed by RAST inhibition titration curves whenever the dust samples con-

tained enough allergen to allow their construction.

Quantitative precipitation inhibition test by laser nephelometry

Quantitative precipitation inhibition is an alternative for quantitative analyses of antigens. Dust extracts with high RAST inhibition values for hen egg white and codfish were chosen for this test.

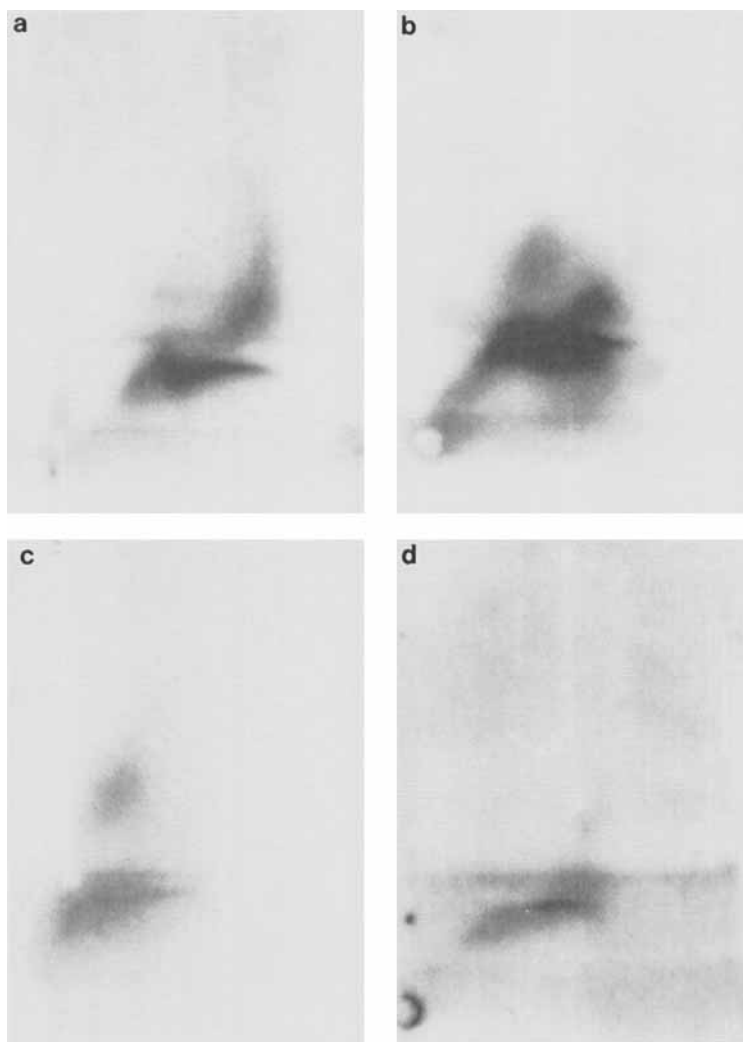


Fig. 2. CRIE films of the dust extracts shown in Fig. 1. The CIE plates were incubated with 0.5 ml serum from hen egg white-allergic patients in 7 ml CRIE buffer followed by incubation with ^{125}I -anti-IgE corresponding to 35,000 cpm/ml. The CRIE plates were then subjected to autoradiography for 2 days.

Direct precipitin experiments were performed by adding increasing amounts of egg white extract or codfish extract to 200 μ l of the relevant rabbit antiserum diluted 1:10 and 1:2, respectively. The reaction was allowed to proceed for 2 h at ambient temperature before turbidity was measured in a laser nephelometer (Behring laser nephelometer, Behring Institute, Marburg, FRG). The signals were read in V and ex-

pressed the concentration of antigen-antibody complex formation (16).

The concentrations of the antigens used for the inhibition experiments were chosen from the optimal equilibrium zone of the standard curves constructed as described above. All solutions were sterile filtered (0.45 μ m) before use. Briefly, aliquots of 300 μ l rabbit antiserum against hen egg white diluted 1:10 in 0.15 M

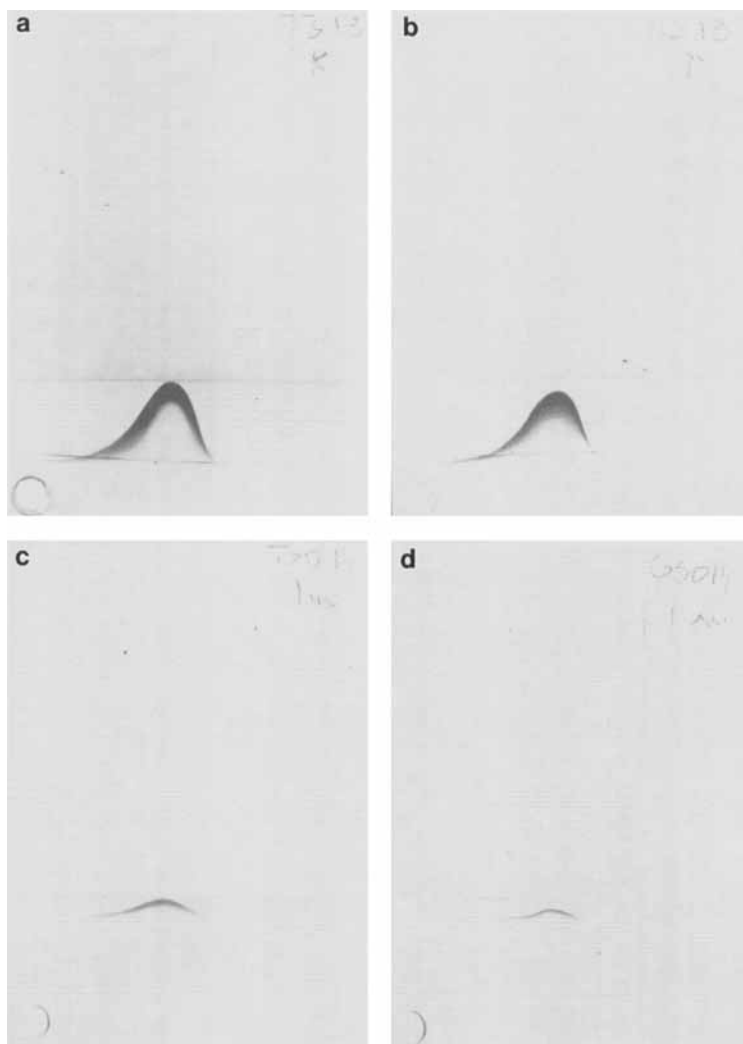


Fig. 3. The patterns obtained in CIE runs of 250 μ g lyophilised dust extracts CF013 (a), SF013 (b), CF014 (c) and SF014 (d). The electrophoresis was performed as described in Fig. 1. The anodic antibody gels contained respectively 10 μ l antiserum against cat dander/cm² (a, b) and 12 μ l antiserum against dog dander/cm² (c, d). In addition the intermediate gels in a and b contained 6 μ l antiserum against human serum albumin/cm².

Table 3

Mean RAST inhibition values in solutions of 2 mg lyophilised dust extracts from 20 carpeted and 20 smooth floors. Arithmetic mean, standard deviation and range are given. Dust from carpeted floors showed the highest RAST inhibition values. With the exception of egg white and codfish ($P < 0.01$), the differences were not significant.

	Carpeted floors ($n = 20$)	Smooth floors ($n = 20$)	<i>P</i> values
Alder	20±17 (0-66)	14±9 (1-39)	0.14
Birch	14±16 (0-57)	9±10 (0-34)	0.16
Timothy	11±6 (0-21)	8±5 (0-17)	0.03
Cat	84±9 (62-92)	78±15 (30-91)	0.04
Dog	71±20 (18-93)	61±20 (30-86)	0.02
Mite	22±15 (4-60)	21±14 (2-55)	0.30
Egg white	42±12 (20-62)	34±9 (21-49)	0.003
Codfish	64±13 (43-83)	48±22 (7-79)	0.003
Mould	76±9 (54-88)	73±9 (58-85)	0.10

NaCl and 0.015 % Tween 20 were added to 150 µl of serial dilutions of lyophilised dust extracts. Dilution buffer without dust extract was used to measure the non-inhibited control value. The reaction was allowed to proceed for 1 h at 37°C. The solutions were refiltered (0.45 µm), and aliquots of 300 µl of the filtrates were added to 50 µl of egg white extract, diluted 1:400. The intensity of the turbidity was measured in V after 3 h.

An identical procedure was followed for codfish antigens. Amounts of 300 µl rabbit anti-serum against codfish (*DIII*), diluted 1:2, were added to 150 µl of serial dilutions of lyophilised dust extracts. After sterile filtration aliquots of 300 µl of the different filtrates were added to aliquots of 50 µl codfish extract 0.2 mg/ml.

Statistics

The Wilcoxon matched-pairs test was used to compare the different parameters in dust from

carpeted and smooth floors. The limit of significance chosen was 0.01. The Wilcoxon two-sample test was chosen to compare the content of house dust mite in dust samples from east and west Norway.

RESULTS

The results obtained showed no significant difference in weight of vacuumed material per m² between carpeted and smooth floors. Dust from carpeted floors contained significantly more lyophilised material and protein per g dust than smooth floors. Mean values of weight of vacuumed material, weight of lyophilised extract and protein concentrations are shown in Table 1.

The number of dust samples from carpeted and smooth floors containing antigens and allergens detected by CIE and CRIE are given in Table 2. The patterns obtained showed that

Table 4

Mean RAST inhibition values in solution of lyophilised dust extracts corresponding to 100 mg dust from 20 carpeted and 20 smooth floors. Dust samples from carpeted floors showed the highest RAST inhibition values. These differences were significant for timothy, cat, dog, egg white, codfish and mould ($P < 0.01$). Arithmetic mean, standard deviation and range are given.

	Carpeted floors ($n = 20$)	Smooth floors ($n = 20$)	<i>P</i> values
Alder	9±14 (0-44)	5±5 (0-18)	0.34
Birch	12±12 (0-39)	6±7 (0-21)	0.08
Timothy	8±7 (0-24)	3±4 (0-13)	0.004
Cat	78±6 (65-87)	66±14 (33-84)	0.001
Dog	69±23 (26-90)	54±16 (27-81)	0.0008
Mite	21±17 (0-58)	14±10 (0-31)	0.04
Egg white	53±11 (31-68)	40±15 (22-63)	0.0006
Codfish	77±14 (44-93)	65±18 (31-87)	0.001
Mould	73±12 (43-87)	59±21 (12-83)	0.003

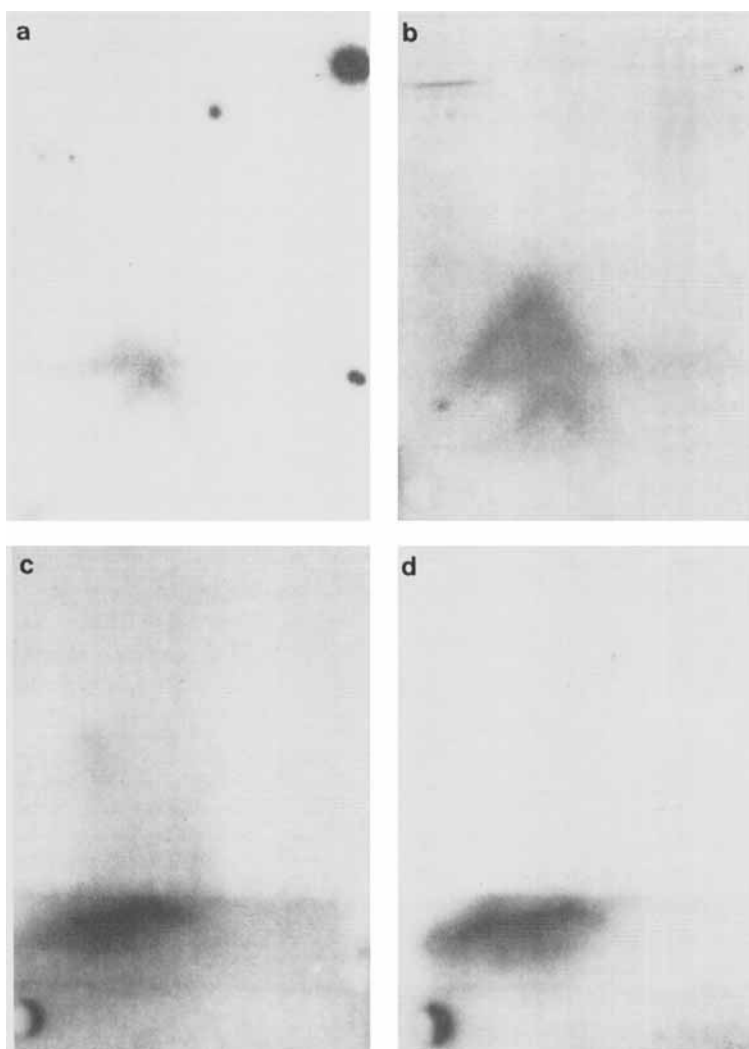


Fig. 4. CRIE of the dust extracts shown in Fig. 3. The CIE plates were incubated with serum from cat dander-allergic patients (a, b) or dog dander-allergic patients (c, d). The autoradiography was performed as described in Fig. 2.

antigens and allergens of cat and dog dander and hen egg white were the most prevalent in the samples investigated. Representative examples of CIE and CRIE runs are shown in Figs. 1–4. In Fig. 1 CIE precipitates of hen egg white are showing only one visible precipitation line. The respective CRIE films are shown in Fig. 2. The CIE precipitate patterns of cat and dog dander are demonstrated in Fig. 3, while their corresponding autoradiographic plates are shown in Fig. 4. The CRIE plates show auto-

radiographic blacking of allergens not detected by Coomassie brilliant blue staining in CIE, demonstrating the sensitivity differences of the two techniques.

The RAST inhibition tests showed, with the exception of hen egg white and codfish allergens, that there were no statistically significant differences in allergen content in identical amounts of lyophilised dust extracts from carpeted and smooth floors. The average RAST inhibition values of 2 mg lyophilised dust ex-

tracts from carpeted and smooth floors are given in Table 3.

RAST inhibition percents of cat, dog, hen egg white, mould, timothy and codfish allergens in aliquots of 100 mg dust were significantly higher for carpeted floors (Table 4). No statistical differences were demonstrated for mite and pollen allergens from birch and alder.

Clear differences in mite concentration in dust from east and west Norway were illustrated. Mean RAST inhibition values for house dust mite in 2 mg lyophilised dust extracts from east and west Norway were 31% and 12%, respectively. This difference was highly significant (the Wilcoxon two sample test, $P \approx 0$).

Precipitation inhibition tests were used to confirm the findings regarding the content of hen egg white and codfish antigens in dust

extracts. When rabbit antiserum was preincubated with 2 mg dust extracts, almost no precipitate was formed between hen egg white (Fig. 5) and codfish antigens (Fig. 6) and their respective rabbit antisera. A dose/response relationship between concentration of dust extracts and inhibition of complex formation was obvious; high concentrations of dust extracts inhibited precipitation of egg white and codfish extracts with rabbit antiserum as measured by nephelometry. Fig. 5 shows results from precipitation inhibition tests including dust extracts and hen egg white. Corresponding results with codfish and dust extracts are shown in Fig. 6.

DISCUSSION

In this study, paired sampling of dust from wall-to-wall carpets and smooth floors in schools allowed comparison of some of the parameters required to evaluate possible disadvantages of carpeted floors. We examined dust from smooth and carpeted floors in classrooms in the same

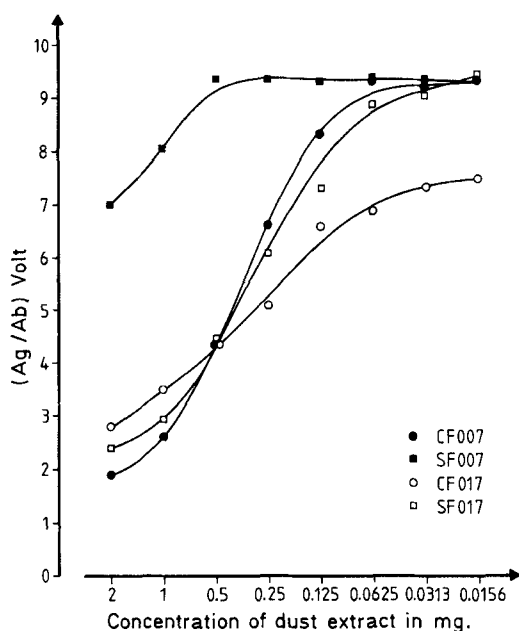


Fig. 5. Quantitative precipitation inhibition, as measured by laser nephelometer, of dust extracts CF007, SF007, CF017 and SF017 in the reaction between hen egg white extract (diluted 1:400, 50 μ l) and rabbit antibodies (diluted 1:10, 300 μ l). The antigen-antibody complex formation is expressed in Volt. The tests were performed in duplicate. Egg white extract with addition of rabbit antiserum without previous incubation with dust extract was used as uninhibited control.

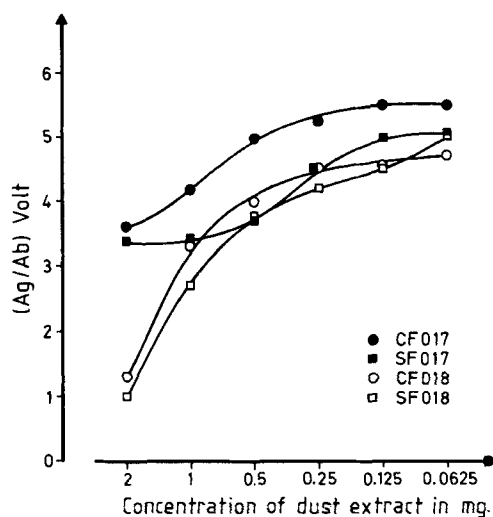


Fig. 6. Precipitation inhibition of dust extracts CF017, SF017, CF018 and SF018. The reaction between codfish extract (diluted 1:54, 50 μ l) and rabbit antibodies against codfish (diluted 1:2, 300 μ l) were inhibited. The inhibiting antigens were dust extracts at an initial concentration of 2 mg. Codfish extract diluted 1:54 in NaCl-Tween with addition of rabbit antiserum without previous incubation with dust extracts was used as a control.

schools to elucidate eventual differences in allergen content. Among the techniques used, RAST inhibition was most useful for quantification and comparison of allergen contents in the different dust samples.

The average content of vacuum cleaner bags in gram per m² was similar for carpeted and smooth floors. This indicates that equal amounts of dust, sand, left overs and other solid constituents were gathered during daily activities in the classrooms regardless of the floor covering.

The CIE and CRIE methods were used for qualitative detection of antigens and allergens in dust samples. Comparison between allergens detected in smooth and carpeted floors by these methods did not elucidate any differences between the floor types. Antigens and allergens of cat, dog and egg white were most prevalent in the samples investigated. The occurrence of the different allergens in classrooms has been explained previously (6). Since the dust samples were collected in December and January they were relatively free of pollen allergens of alder, birch and timothy. The mite content in the dust samples was low, in accordance with another investigation describing low mite counts in schools possibly caused by frequent carpet cleaning, fewer nesting places and a decreased food supply (5). A significantly higher mite content was shown in dust samples collected from schools in west Norway compared with samples from the east. The reason for this difference is probably the higher air humidity at the coastal regions (about 80% relative humidity) being optimal for mite growth (2).

Compared with dust from smooth floors, carpet dust contained more protein as indicated by statistically significant differences both in solid material after lyophilisation and in protein per gram dust. The lyophilised dust extracts from carpets and smooth floors consisted of components with molecular weights over 8,000 daltons and included all potential antigens and allergens. Analyses of equal amounts of lyophilised dust extracts showed no statistically significant differences in content of pollen, animal dander, mite and mould allergens.

Dust from carpeted floors gave more solid matter after lyophilisation than dust from smooth floors. Solutions of lyophilised dust extracts corresponding to 100 mg dust were therefore analysed to obtain an expression of the quantity of allergens in equal amounts of dust from carpeted and smooth floors. More allergens of cat and dog dander, hen egg white, mould, timothy pollen and codfish were demonstrated in dust from carpeted floors. This differs from our first investigation (6), where the type of floor demonstrating the highest levels of allergens varied from school to school. The reasons for the more unambiguous results in this investigation were probably the washing of smooth floors after daily dust sampling by vacuum cleaning and dialysis of the dust extracts resulting in removal of molecules with molecular weights below 8,000 daltons. An additional explanation could be that the carpeted floors were more than 2 years older in this investigation than in our first and thus had retained more macromolecules.

The mean RAST inhibition values for cat, dog, egg white and codfish were 12 to 15% higher in dust from carpeted floors than from smooth floors. Whether these differences in allergen content between carpeted and smooth floors were large enough to cause increase in the amounts of aeroallergens and to be of clinical importance remains to be investigated.

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