Chemical treatment of carpets to reduce allergen: Comparison of the effects of tannic acid and other treatments on proteins derived from dust mites and cats

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Background: *Several chemical treatments have been recommended for reducing mite and other allergen levels in carpets, including the protein-denaturing agent tannic acid (TA).*
Objective: *We evaluated the efficacy of TA and other treatments on mite and cat allergens in carpets within houses. The effects of TA were assessed on Der p 1 and Der f 1, on group II mite allergens, and on the major cat allergen Fel d 1.*
Method: *Carpet treatments tested were benzyl benzoate moist powder, a 3% TA spray, and two carpet cleaners (Host and Capture). Carpets were treated twice and dust samples collected on a biweekly basis for 8 weeks: these samples were extracted in saline solution alone. Additional studies evaluated the effects of TA on 17 carpets. Carpets were treated twice (on days 0 and 28) and samples collected on days 0, 1, 7, 14, 28, and 42. Eighteen carpets were untreated controls. Dust samples were extracted separately in both saline solution and in the presence of 5% bovine serum albumin.*
Results: *Benzyl benzoate and the two carpet cleaners reduced group 1 dust mite allergen concentrations in carpet dust. In addition, benzyl benzoate and TA reduced airborne group 1 mite allergens by more than 64%. Further studies showed that, in keeping with in vitro studies, TA inhibited the assay and bovine serum albumin abrogated this effect. Significant reductions after treatment occurred only for Der f 1 and group 2 dust mite allergens ($p = 0.005$ and $p = 0.035$, respectively). However, for all mite allergens the percentage changes after treatment were significant when compared with untreated carpets ($p < 0.005$ for Der f 1 and group 2 mite, $p < 0.02$ for Der p 1) but not for cat allergen ($p > 0.3$). The results suggested that repeated application of TA was necessary to maintain reduced allergen concentrations.*
Conclusion: *Carpet treatments can reduce mite-derived allergen levels in airborne and carpet dust. However, the effects do not appear to be maintained for long periods, are not dramatic, and are different for different allergens. (J ALLERGY CLIN IMMUNOL 1995;96:325-33.)*

Key words: *Tannic acid, benzyl benzoate, indoor allergens, albumin, ELISA*

In recent years the chemical treatment of carpets has emerged as one possible method of reducing allergen levels in the home. Several studies have demonstrated that indoor allergens are an important risk factor for sensitization and asthma in persons with atopic disease.[1-8] The results have established that the concentration of allergen in dust is a valid index of exposure and imply that levels of allergen in "reservoir" dust correlate with inhaled exposure. Furthermore, threshold levels have been proposed that are relevant both to the risk of sensitization and to the elicitation of symp-

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Abbreviations used
BBS: Borate-buffered saline solution
BSA: Bovine serum albumin
PBS: Phosphate-buffered saline solution
TA: Tannic acid

toms. Group 1 dust mite concentrations of 2 μg/gm or more are considered to increase the risk of sensitization, whereas exposure to more than 10 μg/gm of mite allergen has been associated with increased symptoms.[9, 10] It has been tentatively proposed that cat allergen levels of at least 8 μg/gm should be regarded as a threshold level for both sensitization and asthma.

Allergen avoidance, accomplished either by moving out of the house or by cleaning measures within the house, when carried out rigorously, can significantly improve allergic symptoms.[11-15] The effectiveness of some avoidance measures is undisputed; these include washing bedding in hot water, covering mattresses and pillows with allergen-impermeable covers, vacuuming regularly, and minimizing carpet and upholstered furnishings.[16, 17] In contrast, reports have been conflicting on the effectiveness of various agents marketed to reduce allergens in carpets.[18-26] Such agents include acaricides such as benzyl benzoate, pirimiphos methyl and pyrethroids, and the protein-denaturing chemical tannic acid (TA). Tannic acids are hydrolyzable tannins composed of gallic acid or its condensation product ellagic acid, esterified to the hydroxyl groups of glucose. These gallic acid–containing tannins are alkali labile and are hydrolyzed by gastrointestinal esterases or by tannin acyl hydrolase secreted by fungi, bacteria, and yeast.[27] Tannic acid is marketed either as a 3% spray solution or as a 1% solution in association with a benzyl derivative (DMS solution and Allerbiocid). This treatment has been shown in its various forms to reduce the allergenicity of house dust in vitro and to reduce Der p 1 concentrations in carpets in houses.[24, 28-31]

We have previously reported detailed in vitro studies on the effects of TA on mite and cat allergens.[32] Evaluation of samples of dust collected from carpets in houses treated with TA showed that residual TA redissolves on extraction of dust and inhibits the ELISA, resulting in spuriously low allergen levels. Because TA forms complexes with albumin, we theorized that adding protein to the extraction buffer would abrogate its effects on the ELISA. This proved to be the case, and subsequent assays were carried out on dust samples extracted in the presence of 1% to 5% bovine serum albumin (BSA). Treatment of dust samples with a 3% TA solution in the laboratory produced significant reductions in Der f 1 and Der p 1 allergens when the dust was extracted in the presence of protein. Both group 2 mite allergen and the major cat allergen Fel d 1 were also reduced. However, Fel d 1 was denatured only in samples with baseline concentrations less than 200 μg/gm. TA had no effect in vitro on high levels of cat allergen (>1 mg/gm), and we confirmed that this was a consequence of Fel d 1 blocking the chemical's protein-denaturing properties. The current studies focused on the effects of treatments on carpets in houses. The initial studies evaluated a range of different treatments. We then assessed the effects of TA on the group 1 dust mite allergens Der p 1 and Der f 1, on group 2 dust mite allergens, and on the major cat allergen Fel d 1.

METHODS

Treatment of carpets in houses

All carpet treatment studies were conducted from June to October. Preliminary studies were done to test the effects of four different carpet treatments on allergen levels in carpets. Thirty carpets were selected with more than 2 μg group 1 allergen per gram of dust before treatment. Chemicals used were benzyl benzoate moist powder (Acarosan, Fisons Corp., Rochester, N.Y.), a 3% TA spray (Allergy Control Solution, Allergy Control Products Inc., Ridgefield, Conn.), and two commercially available powders recommended for cleaning carpets: Host (Racine Industries Inc., Racine, Wis.) and Capture (Milliken Chemical, Div. of Milliken and Co., Spartanburg, S.C.). Host contains finely ground, dried corncobs, which confer its hygroscopic properties. Capture is a mixture of a particulate polymeric material, an inorganic salt adjuvant, and an aqueous or organic fluid. Each carpet was randomly allotted to one of the four treatment groups or to an untreated control group. The preparations were applied in accordance with the manufacturer's instructions except for the benzyl benzoate powder, which was left on longer than recommended.[24] All carpet treatments were applied and left overnight, and loose residual material from the surface of the carpet was removed the next day. Carpets were retreated 4 weeks after the initial treatment. Dust samples were collected from treated and control carpets on a biweekly basis for 8 weeks. Dust was collected by vacuuming a 1 m^2 area of carpet for 2 minutes with a portable vacuum cleaner modified to collect dust on a cotton sheet filter. After collection, dust was sieved through a mesh size of 300 μm, and 0.1 gm sieved dust was extracted in 2 ml borate-buffered saline solution (BBS). All samples were

centrifuged to remove particulate matter from the extract and assayed within 2 days for group 1 allergen.

Airborne sampling

Air sampling was carried out in eight houses identified as having high levels of group 1 mite allergen (>20 μg/gm) in carpet dust before treatment. In addition, two uncarpeted rooms were sampled. Samples were collected before treatment (pretreatment) and 2 weeks after the second treatment (posttreatment). A cascade impactor and a glass fiber filter run in parallel were used to detect airborne allergen by methods previously described.[33, 34] Room air was sampled initially for 30 minutes to obtain a baseline airborne mite allergen level. These baseline levels were consistently 0.4 ng/m^3 or less. Air was then sampled during disturbance of floor dust. A vacuum cleaner (Shop-Vac Corp., Williamsport, Pa.) without a filter and dust bag was used to clean the carpet for 15 minutes at a distance of 1.5 m from the air sampler. Air was sampled for a total of 30 minutes, 15 minutes during vacuum cleaning and 15 minutes after switching the cleaner off. The agarose-sorbitol gels and glass fiber filters were eluted in 0.5 and 1.0 ml, respectively, with 1% BSA–phosphate-buffered saline solution (PBS) with polysorbate 20 overnight at 4° C.

Treatment of carpets in houses with 3% TA spray

Further studies were conducted on an additional 17 carpets in houses treated with a 3% TA spray (Allergy Control Solution). Eight of the 17 carpets were on cement slabs, and the remainder were located on ventilated floors (first floor level or higher). TA was applied from a distance of 2 to 3 feet with 12 to 15 sprays to an area of 9 square feet. One 32-ounce sprayer covered 500 square feet. Carpets were treated on day 0, and samples were collected before treatment and on days 1, 7, 14, and 28. Carpets were retreated on day 28 and a final dust sample collected on day 42. Eighteen carpets were untreated controls, and this group comprised nine carpets on cement floors and the same number on ventilated floors. The same protocol was used for the collection of samples from these carpets, except no sample was collected on day 1. Dust samples were collected as described previously and extracted separately in both 5% BSA–PBS–polysorbate 20 and BBS. All samples were assayed for Der f 1, Der p 1, group 2 dust mite allergen, and the major cat allergen Fel d 1 with a two-site monoclonal antibody ELISA. For each carpet the data were analyzed for each allergen with a baseline level of at least 1 μg per gram of dust. Eleven carpets were analyzed for Der f 1, 10 for Der p 1, 12 for group 2 dust mite, and 13 for cat allergen. Numbers of control carpets with significant allergen levels were 11 for Der f 1 and Der p 1, 12 for group 2 mite, and 15 for Fel d 1. Carpets with low baseline allergen concentrations (<1 μg/gm) maintained these levels throughout the study.

ELISA for the quantitation of dust mite and cat allergens

The allergen content of carpet dust and airborne samples was assayed by a two-site ELISA with monoclonal antibodies specific for two nonoverlapping epitopes on Der p 1, Der f 1, group 2 mite, and Fel d 1 allergens.[35-37] A comparison was made between allergen concentrations obtained after extraction of the same dust sample in BBS and in 5% BSA PBS–polysorbate 20. Extracting samples in BBS or PBS did not influence values obtained.

Statistical analysis

The allergen concentrations present in carpets on days 0 and 42 were expressed as a geometric mean and a range. Student's paired t test was used to compare changes in allergen concentrations over time, and Student's t test for independent variables was used to compare percentage changes in allergen in treated carpets with those in control carpets.

RESULTS

Preliminary studies on carpet treatments and airborne allergen levels

Four different chemical treatments were applied to 30 carpets (Table I). Dust samples were collected every 2 weeks. Results show the percentage change in both mite allergen concentration and the total quantity of allergen recovered from carpets at 2 and 8 weeks after the initial treatment. In accordance with previous reports, benzyl benzoate applied for 12 hours significantly reduced group I mite allergen.[24] Carpets treated with TA showed a more marked decrease in allergen. However, these dust samples were extracted in BBS, and subsequent work showed that values obtained by such an extraction method may not reflect true allergen levels. Interestingly, both Host and Capture cleaners also caused a decrease in allergen concentration. A surprising effect of treatment with Capture cleaner was the significant increase in total allergen recovered. In a second series of experiments airborne measurements were conducted in eight of the houses. All airborne samples were extracted in the presence of 1% BSA. Results showed a marked decrease (>64%) in airborne mite allergen during disturbance after treatment with either benzyl benzoate or TA (Table II). Airborne mite allergen was undetectable in undisturbed conditions and was unmeasurable in two uncarpeted rooms even during disturbance. Treatment with TA caused mean percentage reductions of 64% for Der p 1 and 85% for Der f 1 when sampling during disturbance (data not shown). TA appeared to cause a greater decrease in group 1 allergen levels when pretreat-

TABLE I. Group 1 mite allergen levels in carpet dust after chemical treatment*: Change from pretreatment levels

	Mean % change in concentration of allergen*			Mean % change in total allergen recovery*		
	2 wk	8 wk	Average change during wk 2-8	2 wk	8 wk	Average change during wk 2-8
Benzyl benzoate (n = 8)	−69	−79	−75	+84	−60	+4.1
Capture cleaner (n = 6)	−63	−60	−54	+435	+460	+528
Host cleaner (n = 5)	−56	−65	−63	−36	−64	−42
3% Tannic acid (n = 6)†	−94	−73	−84	−95	−78	−72
Control (n = 5)	+39	+45	+76	−8.4	−2	−12

Samples were obtained at biweekly intervals and extracted in BBS.
*Group 1 allergen concentration measured in micrograms per gram of dust. Total allergen recovery calculated as Concentration (microgram per gram) × Weight (grams) of Sieved Dust.
†Percent reduction was probably exaggerated by effect of TA on assay.

TABLE II. Effects of carpet treatment on total airborne group 1 mite allergen

	Total airborne group I mite allergen (ng/m³)*									
	3% Tannic acid				Benzyl benzoate		Host	Capture	Uncarpeted floors	
House	BV	MR	AT	RS	WM	RC	NS	RT	MW	TM
Before treatment										
Undisturbed	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
Disturbed	20.2	53.1	190	184	17.8	377	47.2	21.2	<0.8	<0.8
After treatment†										
Undisturbed	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	—	—
Disturbed	7.2	15.8	9	42.9	4.4	38.4	32.6	47.4	—	—
Reduction (%)	64	70	95	77	75	90	31	—	—	—

Values shown are the mean of airborne dust mite collected on the cascade impactor and parallel filter. Letters are initials of house owners.
*Air was sampled for 30 minutes in undisturbed conditions to obtain a baseline value. Air was then sampled during 15 minutes of disturbance; a vacuum cleaner without a filter was used to clean the carpet and was kept 1.5 m away from the sampler.
†Two weeks after second treatment.

ment levels were highest. The data is also shown for single houses treated with Host and Capture cleaners where moderate changes in airborne mite allergen levels occurred in parallel with changes in carpet dust. These results suggest that treatment of carpets with TA or benzyl benzoate can decrease the quantity of mite allergen that becomes airborne during vacuum cleaning.

Effects of TA on the ELISA

In light of the previous in vitro data on TA, we investigated the true effects of this chemical on allergen levels in houses. Carpet dust samples were extracted separately in BBS and 5% BSA–PBS–polysorbate 20. Mean percentage reductions in groups 1 and 2 dust mite allergen and cat allergen were compared after extraction of dust by the two different methods. Only houses where sufficient dust was collected for extraction by the two methods (i.e., ≥0.1 gm) were compared. Results confirmed that TA in dust extracts in the absence of protein can inhibit the ELISA, resulting in falsely low allergen levels after treatment (Table III). The largest discrepancy in the values for mean percentage reduction occurred for Der p 1 and the smallest for Der f 1 and group 2 allergens. The mean percentage changes for untreated carpet dust samples extracted in the absence of protein were not significantly different from those extracted with BSA. These findings are in keeping with our in vitro studies. For reasons that are not clear, residual TA in samples taken from carpets on concrete slabs appeared to exert a greater inhibitory effect on the ELISA than TA in extracts from carpets on ventilated floors.

TABLE III. Effect of protein (BSA) in the extraction buffer on the measurement of allergen in dust from carpets treated with 3% tannic acid

	BBS			5% BSA–PBS–polysorbate 20		
	Mean allergen level (μg/gm)*		Mean	Mean allergen level (μg/gm)*		Mean
Location of carpet	Day 0	Day 42	decrease (%)	Day 0	Day 42	decrease (%)
Ventilated floor						
Der p 1 (n = 2)	8.8	18.7	−19	7.8	20.6	+30
Der f 1 (n = 4)	16.8	0.3	−95	21	3.2	−77
Group 2 (n = 5)	13.5	0.5	−84	10.4	1.1	−68
Fel d 1 (n = 4)	71	46.3	−35	76.2	65.8	−23
Concrete slab						
Der p 1 (n = 5)	24.5	9.7	−87	25.9	26.2	−21
Der f 1 (n = 5)	14.2	0.2	−95	20.7	9.5	−62
Group 2 (n = 5)	45	2	−97	36.4	3.9	−76
Fel d 1 (n = 5)	76.2	8.1	−70	87.9	33.5	−31

n, Number of houses compared.
*Values are the mean allergen levels immediately before the first application of TA (day 0) and 2 weeks after the second application of TA (day 42). All houses had pretreatment levels greater than 1 μg/gm.

Effects of TA treatment on allergen levels in carpet dust

The effect of TA on Der p 1, Der f 1, group 2 mite allergen, and Fel d 1 was examined when all samples were extracted in the presence of 5% BSA. Treated carpets containing significant levels of one or more allergen (≥1 μg/gm) were compared both before (baseline, day 0) and after treatment (day 42) with untreated control carpets (Table IV). Data are shown both for carpets on ventilated floors and for those on concrete slabs, because of previous concerns that TA was less effective on basement carpets and that treatment resulted in staining of carpets that were damp. However, in the current study the differences between these sets of carpets were modest and the results were combined for the purpose of statistical analysis. Significant reductions in both Der f 1 and group 2 allergen occurred after two applications of TA (p = 0.005 and 0.035, respectively). All carpets showed a decrease in Der f 1 after treatment, and 11 of 12 showed reductions in group 2 concentrations. Seven of 10 treated carpets showed decreases in Der p 1 concentration, but this effect was not significant. TA had inconsistent effects on cat allergen levels in carpets on ventilated floors. However, a mean percentage reduction in Fel d 1 of 40% occurred in carpets on concrete slabs. The percentage changes for all allergens after treatment were significantly different from those of the control carpets ($p < 0.02$) with the exception of cat allergen ($p > 0.3$). During the study allergen levels in untreated carpets tended to increase, and this was most apparent for Fel d 1 allergen, which showed a mean percentage increase of 90%, and Der p 1, which increased by 71%. These results demonstrate that TA exerted a significant effect on dust mite allergens when compared with untreated control carpets. Although TA had an effect on cat allergen in carpets on concrete slabs, the overall effect when the percentage change of Fel d 1 in 13 treated carpets was compared with 15 control carpets was not significant.

Finally, we studied the time course of action of TA on Der f 1 and group 2 mite allergens. Significant reductions occurred 24 hours after the first application of TA (p = 0.01 for Der f 1 and p = 0.03 for group 2 dust mite). Allergen levels remained at less than baseline levels with a slight increase at day 14 (Figs. 1, *A* and 2, *A*). Allergen levels then decreased and continued to decrease 2 weeks after the second treatment (day 42). In contrast, allergen concentrations in untreated control carpets either remained unchanged or actually increased (Figs. 1, *B* and 2, *B*).

DISCUSSION

This study demonstrated that TA exerts an effect on several major indoor allergens. Although in our initial carpet treatment studies all samples were extracted in the absence of protein, several interesting observations came to light: not only did

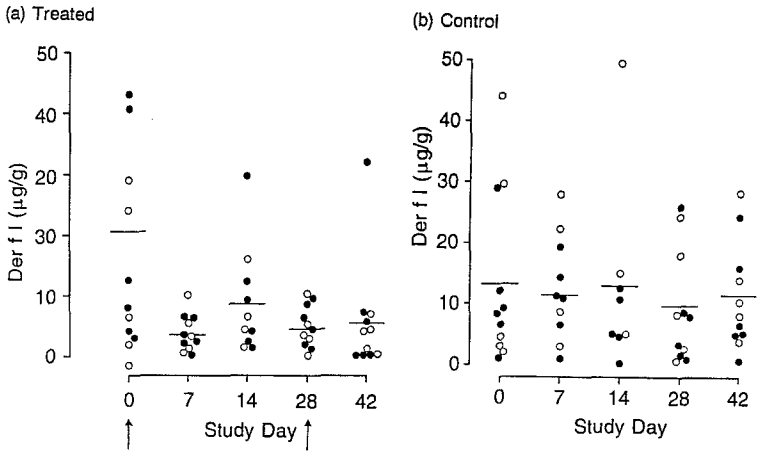

FIG. 1. Changes in concentration of Der f 1 in carpets on ventilated floors (○) and concrete slabs (●). Carpets were either treated with TA **(a)** or untreated control carpets **(b)**. Number of treated and untreated carpets with more than 1 μg/gm Der f 1 allergen on day 0 was 11; where fewer than 11 values are shown, dust samples were not obtained (e.g., day 14 [n = 10] for treated carpets and days 7 and 14 [n = 10 and n = 8, respectively] for control carpets). *Horizontal bar* represents arithmetic mean.

TABLE IV. Allergen levels in treated and control carpets

	Der f 1		Der p 1		Group 2		Fel d 1	
Location of carpets	**Treated***	**Control**	**Treated**	**Control**	**Treated†**	**Control**	**Treated**	**Control**
Ventilated floor								
n	5	5	4	4	6	5	6	6
Day 0								
Geometric mean	13.6	8	2.5	4.5	4.2	7.8	10.2	10.7
Range	2-51.3	2-43.8	1.1-13.3	1.5-16.9	1.3-40	2.4-22.7	1-161	1.1-800
Day 42								
Geometric mean	2.6	10.5	1.6	7.1	0.7	9	9.1	16.2
Range	0.6-6.4	3.7-28.5	0.3-27.9	3.2-23.8	0.2-2.5	5-15	0.2-210	0.9-1125
Concrete slab								
n	6	6	6	7	6	7	7	9
Day 0								
Geometric mean	11.4	7.4	6	4.5	17.4	6.5	14.5	14.4
Range	3-43.2	1-29	1.1-114	1.5-10.5	2.6-126	1.5-22.6	1.4-338	1.2-290
Day 42								
Geometric mean	2.8	6.6	2.6	6	1.7	7.5	5.8	21.2
Range	0.5-33.8	1.1-24.4	0.2-118	0.4-16.9	0.2-7.9	1.6-33	0.1-86	1-613
Mean % change for all carpets	−73[a]	+26	−28[b]	+71	−76[a]	+16	+23[c]	+90

Values are for carpets with allergen levels greater than 1 μg/gm on day 0.
Analysis for day 42 versus day 0 for all carpets: *p = 0.005. †p = 0.035.
Percentage changes of treated carpets were compared with control carpets:
[a]$p < 0.005$.
[b]$p < 0.02$.
[c]$p > 0.03$.

benzyl benzoate cause a reduction in group 1 allergen concentrations, but Host and Capture cleaners also caused a similar outcome. Benzyl benzoate is said to act not only by virtue of its acaricidal effects but also by "cleaning" and possible dilution effects. Host cleaner may act by virtue of its hygroscopic properties, resulting in a less favorable environment for mite growth. The polymeric particle sizes of Capture cleaner range from 37 to 105 μm for maximum penetration of carpet

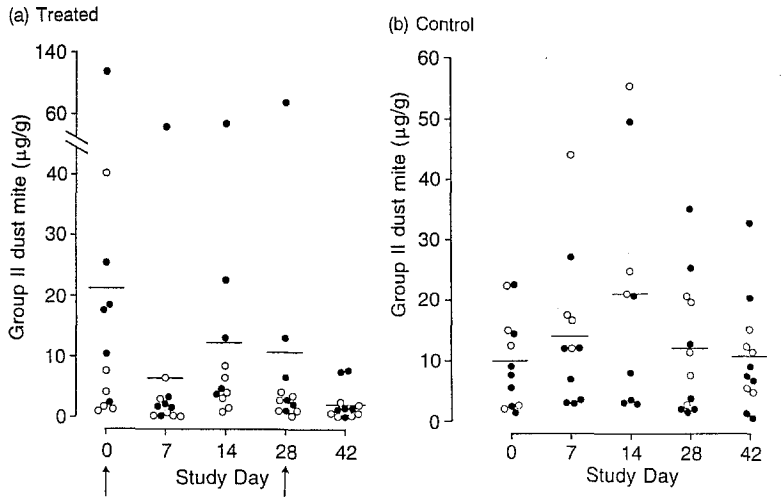

FIG. 2. Changes in concentration of group 2 mite allergen in carpets on ventilated floors (○) and concrete slabs (●). Carpets were either treated with TA **(a)** or untreated control carpets **(b)**. Number of treated and untreated carpets with more than 1 µg/gm group 2 mite allergen on day 0 was 12; where fewer than 12 values are shown, dust samples were not obtained (e.g., day 14 [n = 11] for treated carpets, and days 7 and 14 [n = 11 and n = 9, respectively] for control carpets). *Horizontal bar* represents arithmetic mean.

fibers. The reduction in allergen concentration and increase in total allergen recovery is more likely to be attributable to a cleaning effect of this substance rather than an effect on mite growth.

Airborne sampling confirmed the absence of mite allergen in undisturbed conditions. To study the potential for airborne allergen exposure, we used a technique of vigorous disturbance. Previous attempts to study airborne mite allergen during normal domestic activities have not given consistent results. All airborne samples were extracted in the presence of protein buffer, making it possible to compare directly reductions obtained by different carpet treatments. Comparable reductions of group I mite allergen occurred for TA and benzyl benzoate, suggesting that both treatments can reduce exposure. Because only a single airborne sample was obtained after treatment with Host and Capture cleaners, it is difficult to draw any distinct conclusions regarding the effects of these agents on airborne allergen. However, changes in airborne levels after treatment corresponded with changes in allergen in the carpets. The increase in airborne mite allergen after treatment with Capture cleaner may be attributable to small particles of polymer that become associated with allergen and remain in the carpet for long periods. We assume that these particles become airborne on disturbance, resulting in increased airborne allergen levels.

Because of the current widespread use of TA, we carried out further experiments on this agent to resolve its effects on indoor allergens. In keeping with our previous findings, residual TA in treated carpet dust samples is redissolved and inhibits the ELISA. This results in spuriously low allergen levels and exaggerated reductions after treatment (Table III). In some houses this effect was more pronounced than others, and this may reflect variations in the total protein content of different dusts. Tannin-protein complex formation is governed by the relative amounts of TA and protein and also by the mix of proteins present. It is possible that carpets on concrete slabs have increased allergen concentrations but less total protein than carpets on ventilated floors, which are located in the primary living space and exposed to more traffic. This may explain why TA in samples from carpets on concrete floors exerted a greater effect on the ELISA than that in samples from carpets on ventilated floors.

TA was more effective at reducing the concentrations of Der f 1 and group 2 mite allergens than at decreasing the levels of Der p 1 and cat allergens. The propensity of different proteins to be denatured by tannins is dependent on several factors including the size of the peptide, its tertiary structure, and the pH of the reaction conditions.[38-41] Tannin has been reported to bind especially tightly to proline-rich proteins, which are strong hydrogen bond acceptors.[42] Der f 1 and Der p 1 are homologous forms of a fecally derived hydrolytic cysteine protease. One possible explana-

tion for the apparent difference of these allergens in susceptibility to denaturation is the presence of an extra proline residue at position 98 in Der f 1. However, neither peptide has a particularly high proline content (3.6% for Der p 1 and 4.1% for Der f 1) and that of the group 2 mite allergens is only marginally higher (6.2%). Group 1 and group 2 dust mite allergens differ in their conformational stability, and this may also contribute to variations in susceptibility to the actions of TA.[43]

In contrast to our in vitro studies, cat allergen in carpets was not significantly denatured by TA regardless of the initial concentrations. We previously showed that high levels of cat allergen can actually inhibit the action of TA on other allergens.[32] Fel d 1 is a glycoprotein containing approximately 20% carbohydrate. It has been reported that some enzymes rich in carbohydrate demonstrate resistance to hydrolyzable tannins.[44] It was proposed that the carbohydrate coating protects the polypeptide backbone, making it less accessible to tannins. However, more recent studies have shown that certain proline-rich glycoproteins have very high affinity for tannins.[45] More work is needed to elucidate the role of carbohydrate residues in interactions between TA and cat allergen, and to assess how such interactions may affect other allergens present. Finally, work conducted in the early 1940s suggested that TA can precipitate ragweed allergen without reducing its antigenicity.[46] A similar interaction between TA and cat allergen could explain why cat allergen is not denatured. The complex nature of the interaction of TA with proteins may explain why denaturation of cat allergen can be demonstrated in vitro but when TA is tested on carpets the results are inconsistent.

Allergen levels in untreated carpets generally increased throughout the TA study. This is not surprising because sampling was conducted from the final week of August to the end of the first week of October. Our results do not resolve the length of action of TA, but they suggest that one application is not sufficient to maintain low allergen concentrations (Figs. 1, *A* and 2, *A*).

The results confirm that treatment with either an acaricide or a protein-denaturing agent can reduce the concentration of mite-derived allergens in carpets. However, the reductions achieved are not universal, are not of long duration, and are certainly not as effective as removing carpets. Chemical treatment of carpets may be a useful element in a protocol designed to reduce exposure to mite allergens if carpets cannot be removed. However, it is clear that repeated treatment is required. In addition, our results suggest that more work is needed to identify alternative treatments for reducing the concentration of allergens in carpets.

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