Chemical treatment of carpets to reduce allergen: A detailed study of the effects of tannic acid on indoor allergens

Judith A. Woodfolk, MB, ChB, Mary L. Hayden, RN, Jeffrey D. Miller, MD, Gail Rose, BS, Martin D. Chapman, PhD, and Thomas A. E. Platts-Mills, MD, PhD *Charlottesville, Va.*

*Tannic acid (TA), a protein-denaturing agent, has been reported to reduce allergen levels in house dust and is marketed for that purpose as 1% and 3% solutions. We investigated the effects of TA on dust allergens by using monoclonal antibody-based ELISAs for mite (*Der p *I,* Der f *I, and group II) and cat (*Fel d *I) allergens. Initial studies confirmed that TA reduced allergen levels in carpet dust. However, when dust samples from treated carpets are extracted in saline solution, residual TA redissolves and may interfere with the assessment of allergens. In the laboratory, concentrations of TA as low as 0.1% inhibited the assays, but this effect may be prevented by addition of 5% bovine serum albumin (BSA). After treatment of dust samples in the laboratory with 3% TA, the apparent reductions in* Der p *I and* Der f *I levels were 89% and 96%, respectively, but when the samples were extracted in 5% BSA the reductions were 74% and 92%. Similar effects were seen with dust samples from carpets treated with TA. In an extreme case in which a carpet had been repeatedly treated with TA, the apparent concentration of* Der p *I was <0.05 μg/gm without BSA and 2.1 and 8.4 μg/gm when extracted in the presence of 1% and 5% BSA, respectively. Our testing of the ability of TA to denature* Fel d *I demonstrated an 80% reduction in allergen, but only in samples with an initial concentration of less than 200 μg* Fel d *I/gm dust. In samples with high levels of* Fel d *I (~1 mg/gm) TA had little effect. The interpretation of this was that* Fel d *I itself could block the effects of TA. In keeping with this,* Fel d *I inhibited the effect of TA on* Der p *I. The results confirmed the profound denaturing effects of TA, but demonstrated that high levels of protein blocked the effect of TA on dust allergens. In addition, without added protein, residual TA in dust samples could interfere with the assay of allergens in vitro. (J Allergy Clin Immunol 1994;94:19-26.)*

Key words: *Tannic acid, dust mite allergen, cat allergen, ELISA, inhibition, albumin*

During the last 20 years increasing evidence has emerged that indoor allergens play an important role in perennial rhinitis, atopic dermatitis, and asthma.[1-5] Logically, these results have suggested that avoidance measures inside houses should be a primary form of treatment for these patients. Several studies have demonstrated that effective control of dust mite allergen levels can decrease symptoms of asthma and decrease bronchial reactivity.[6-13] Measures taken to reduce allergens in houses have included washing bedding in hot water, encasing mattresses and pillows in allergen-impermeable covers, using vacuum cleaners and air filters, removing carpets, and reducing upholstered furnishings. In addition, various chemical

Abbreviations used

BBS:	Borate-buffered saline solution
BSA:	Bovine serum albumin
mAb:	Monoclonal antibody
PBS:	Phosphate-buffered saline solution
PBS-T:	Phosphate-buffered saline solution with Tween 20
TA:	Tannic acid

From the Division of Allergy and Clinical Immunology, Department of Medicine, University of Virginia Health Sciences Center, Charlottesville, Va.

Supported by NIH Grant No. AI-20565 and by an Educational Grant from Allergy Control Products (for the training of J.A.W.).

Presented at the 50th Anniversary Meeting of the American Academy of Allergy and Immunology, Chicago, Ill., March 1993.

Received for publication April 6, 1993; revised July 22, 1993; accepted for publication July 28, 1993.

Reprint requests: Thomas A. E. Platts-Mills, MD, PhD, Division of Allergy and Clinical Immunology, Department of Medicine, Box 225, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

0091-6749/94 $3.00 + 0 **1/1/55432**

methods have been applied in an attempt to eliminate allergen from carpets, upholstery, and bedding. Chemicals used include liquid nitrogen; acaricides (pirimphos methyl, natamycin, and benzyl benzoate); tannic acid (TA), as a 1% or 3% solution; and also DMS solution (Allersearch Laboratories, Division of Alkaline Corp., Oakhurst, N.J.), which contains 1% TA and a benzyl derivative.[14-20]

TA is a naturally occurring substance found in the bark and fruit of many plants, notably the oak species. There are multiple types of tannins, and they are generally divided into two groups: the condensed group, derivatives of flavenols, and the more important hydrolyzable tannins, esters of a sugar with one or more trihydroxybenzene carboxylic acid residues. TA gives insoluble precipitates with albumin and produces a bluish black color with ferric salts. Industrial uses of TA include the manufacture of imitation horn and the tanning of leather. Therapeutically it has been used as an astringent. Several studies have shown that tannins can inhibit digestive enzymes.[21-23] The protein-denaturing properties of TA are well recognized, and for this reason it has been marketed for the eradication of dust mite and cat allergens in house dust. Green et al. showed that a 1% TA solution completely abolished the allergenicity of house dust used in skin tests and theorized that washing bedding and other fabrics in a 1% TA solution would reduce allergens.[24] Further studies by the same group involving the application of DMS solution showed that the solution both killed dust mites and reduced the allergenicity of house dust.[20, 25]

In recent years it has been possible to accurately measure the concentration of certain major allergens in house dust by means of the ELISA.[26-29] Allergens that may be assayed by this method include those produced by house dust mites of the genus *Dermatophagoides;* the major cat allergen *Fel d* I and cockroach allergens *Bla g* I and *Bla g* II. Researchers assessing the allergen content of dust after treatment with TA have used both skin-prick test bioassays and ELISA.[20, 24, 25, 30] Because TA is a chemically active substance capable of denaturing proteins, it is reasonable to assume that it would interfere with the ELISA. However, no investigation of this effect has been published. Preliminary work showed that extracts of treated carpet dust formed a bluish black precipitate when ferric chloride was added. This indicated that TA may be resolubilized when dust from treated carpets is extracted in saline solution. Therefore it is possible that residual dried TA in dust from treated carpets may interfere with the ELISA, resulting in falsely low allergen levels. Attempts to remove TA from extracts by dialysis were unsuccessful. We investigated the ways in which TA interferes with the ELISA and developed methods of avoiding these effects.

METHODS

Sample collection in houses

Six carpets were treated with a 3% TA spray solution (Allergy Control Solution; Allergy Control Products, Inc., Ridgefield, Conn.). The treatment was applied from a distance of 2 to 3 feet with 12 to 15 sprays per square yard. One 32-ounce sprayer covered 500 square feet. The carpet was re-treated 4 weeks after the initial treatment. Dust samples were collected from the carpet after treatment with a portable vacuum cleaner modified to collect dust on a cotton sheet filter. Dust was collected by vacuuming a 1 m^2 area of carpet for 2 minutes. After collection, dust was sieved through a mesh size of 300 μm, and 0.1 gm sieved dust was extracted in 2 ml of borate-buffered saline solution (BBS). Samples were also extracted in 30% bovine serum albumin phosphate-buffered saline solution with Tween 20 (BSA PBS-T). Preliminary work had shown no significant difference in allergen levels obtained for samples extracted in BBS or PBS alone. All samples were centrifuged to remove dust from the extract. Carpet dust extracts were assayed within 2 days or stored at −20° C.

Treatment of dust samples with TA in the laboratory

Dust samples were obtained from vacuum cleaner bags from 11 untreated houses. Dust was sieved and divided into four 0.1 gm aliquots from each house. A volume of 250 μl of 3% TA solution was added to two of the samples and 250 μl of distilled water to the two remaining samples as controls. Dust samples were incubated for 4 hours at room temperature. The treated samples and control samples from each house were extracted, either in 2 ml of BBS or in 5% BSA PBS-T by rotating the dust samples overnight at 4° C. Extracts were then assayed for group I and group II house dust mite allergen and the major cat allergen *Fel d* I by a two-site monoclonal ELISA.

ELISA for the quantification of dust mite, cat, and cockroach allergens

A two-site ELISA, using monoclonal antibodies (mAb) specific for two non-overlapping epitopes on *Der p* I, *Der f* I, group II mite, and *Fel d* I allergens, was used to assay the allergen content of dust and airborne samples and to study the effect of TA on the ELISA.[26, 27, 29] An ELISA for *Bla g* I and *Bla g* II, with

TABLE I. Effect of protein (BSA) in the extraction buffer on group I mite allergen concentrations in dust obtained from six carpets treated with 3% TA*

House	*Der p* I (μg/g)		*Der f* I (μg/g)	
	BBS	30% BSA PBS	BBS	30% BSA PBS
R.S.	<0.05	9.4	ND	ND
B.B.	1.8	1.4	1	0.7
P.K.	0.1	29.8	0.1	10.7
M.R.	5.6	5	20.5	36.4
P.T.	0.9	35.3	0.1	0.2
B.V.	0.1	0.4	0.1	18.8

ND, Not done.
*All values shown indicate allergen content 2 weeks after second application of tannic acid to carpets in houses.

polyclonal rabbit antibodies used as the second antibody, was done to study the effect of TA on the cockroach assay.[28] Control curves for each standard allergenic extract were established in 1% BSA PBS-T diluent in parallel with each assay. In addition, control curves were set up in diluents containing 0.01% to 3% TA in 0.25% BSA PBS-T to study the effect of TA on the ELISA.

RESULTS

Effects of TA on the ELISA

During preliminary studies in houses, we observed that airborne mite allergen was detectable in one house (designated R.S. in Table I) that had been treated twice with TA, even though little or no group I mite allergen was detectable in dust from the carpet. A possible explanation for this phenomenon was that residual TA in the carpet dust was influencing the result of the ELISA. Therefore we examined the effects of TA on the ELISA by establishing control curves for group I and II mite, cat (*Fel d* I), and cockroach (*Bla g* I and *Bla g* II) in a diluent containing 0.01% to 3% TA. Standard extracts were incubated with the diluent in the plate for 1 hour. The effect of TA on control curves for *Der f* I, *Fel d* I and *Bla g* I immunoassays is shown in Fig. 1. TA significantly affected each assay at a concentration of 0.1% but exerted little effect at 0.05%. Therefore it would be necessary to dilute a 3% TA solution 60-fold or more to avoid direct effects on the assay. Because the exact concentration of TA in treated carpet dust extracts cannot be determined and because it is not possible to assay dust samples with low concentrations of allergen at higher dilutions, it would not be practical to dilute all extracts in such a way. TA exerted a greater effect at a concentration of 0.1% on the ELISA for *Fel d* I than on that for *Der f* I (Fig. 1). A lateral shift of each control curve occurred at a concentration of 0.1% TA, corresponding with an apparent reduction in allergenicity of 59% for *Der f* I and approximately 82% for *Fel d* I and *Bla g* I. Initial studies demonstrated that the effect of TA on the ELISA could not be attributed to acidic pH; the 0.1% TA diluent gave a pH of 7.0, compared with 7.3 for buffer alone, and adjustment of the pH to 7.3 by using 1 mol/L sodium hydroxide did not reduce the effect of TA on the control curves. To understand the mechanism by which TA affected the ELISA, the *Der f* I extract used for the standard curve was treated with 0.01% to 3% TA in the laboratory for 1 hour. Protein buffer (10% BSA PBS-T) was added to all samples after 1 hour, and these were assayed the same day. No reduction in allergenicity of the extract occurred after 1 hour with concentrations of TA that had been shown to exert an effect on the standard control curves (i.e., 0.1% to 0.15%). However, extract incubated with 3% TA showed >98% denaturation. To test whether TA interfered with the coating mAb, plates coated with the anti-*Der f* I mAb (6A8) were washed and blocked with 1% BSA PBS-T by the usual method before incubating for 1 hour with 0.01% to 3% TA solutions in PBS. Control curves for *Der f* I using plates treated with TA showed little or no shift of the curve, suggesting that TA did not act directly on the coating mAb. These results implied that the effect of diluted TA on ELISA control curves was not attributable to denaturation of allergen or a direct effect on the coating mAb.

Testing dust extracts from carpets treated with TA confirmed the presence of residual TA. The pH was 4.9, compared with 6.8 for untreated controls, and the addition of ferric chloride gave a strong blue color, indicating the presence of TA in the extract. This suggested that TA in the dust

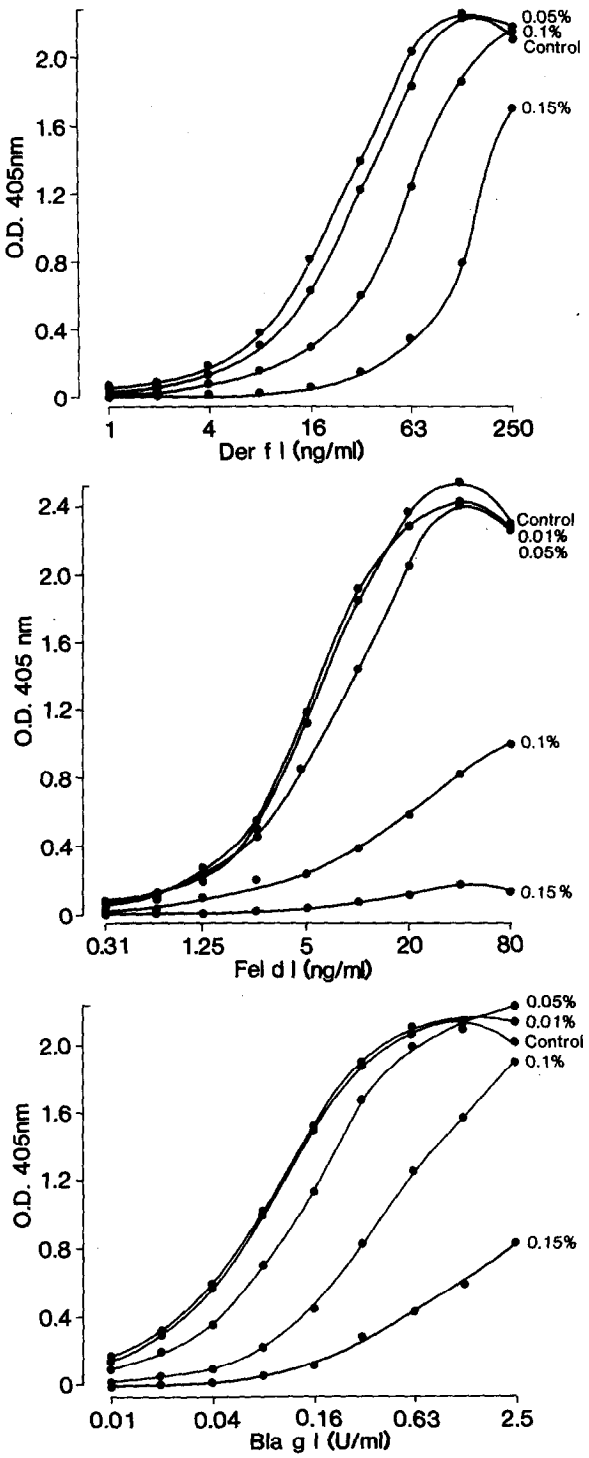

FIG. 1. The effect of different concentrations of TA on immunoassays for *Der f* I, *Fel d* I, and *Bla g* I.

may not only influence the assay but may also act to denature allergen after extraction of dust samples. In previous studies we extracted floor or upholstered furniture dust samples in BBS and airborne samples in 1% BSA PBS-T. Albumin was routinely added to the eluent for airborne samples because of the very low levels of airborne protein.[31, 32] Because it is known that TA binds to albumin, we tested whether albumin could block the effects of TA on the ELISA. Extraction of floor dust from the house described above (R.S.) in buffered saline solution alone resulted in apparent levels of group I mite allergen of <0.05 μg/gm, but extraction in the presence of 1% and 5% BSA gave levels of 2.1 and 8.4 μg/gm, respectively. Extracting this dust sample in BSA concentrations from 5% to 30% resulted in only a minor additional increase in apparent allergen content (i.e., $<10\%$ further increase).

Dust samples from six carpets treated with 3% TA spray were extracted in buffered saline solution or saline solution containing 30% BSA to compare allergen levels obtained by ELISA. Four extracts showed higher levels of *Der p* I, *Der f* I, or both, when protein was present (Table I). Despite the large difference in the effect of BSA on different dust samples, the results for each sample were consistent when repeated on separate occasions. When some of the treated samples were extracted in saline solution without protein, the undiluted extract showed dramatic inhibition of the assay. This inhibition was abolished when the same samples were extracted in the presence of BSA. Thus it appeared that the presence of BSA blocked the effects of TA on the ELISA and also any denaturation of allergen by TA that might have occurred after extraction.

Treatment of dust samples in the laboratory

Carpet dust samples were treated for 4 hours with 3% TA, and the dust was then extracted in either BBS or 5% BSA PBS-T. Treated samples extracted in saline solution alone showed an 89% reduction in *Der p* I, whereas those extracted in the presence of 5% BSA showed only a 74% reduction (Table II). Surprisingly, the results for *Der f* I showed little difference between the two extraction buffers; samples extracted in saline solution alone showed a 96% reduction in allergen, compared with 92% in the presence of protein. However, it appeared that the reason for this was that TA had a greater denaturing effect on *Der f* I. TA exerted less effect on group II allergen, with reductions ranging from 43% to 94% (71% mean reduction), and allergen values were not influenced by the presence of protein. The largest percentage reductions in allergen levels were seen for group I and group II mite allergens

TABLE II. Effect of protein (BSA) in the extraction buffer on group I mite allergen concentrations in dust treated with 3% TA in the laboratory

	Method of extraction			
	BBS		5% BSA PBS	
Sample	Control	TA	Control	TA
Der p I content (μg/gm):				
R.S.	57.3	0.2	46.1	4.6
U.N.	1.6	<0.05	1.9	0.1
M.G.	4.6	1.7	5.2	2.9
H.J.	8.4	<0.05	7.6	2.5
Mean	18	0.5 (89%)	15.2	2.5 (74%)
Der f I content (μg/gm):				
M.S.	46.4	2	45.8	2
S.M.	13.1	0.2	12.7	1.2
B.C.	9.7	0.8	10.4	1.2
R.S.	5.1	<0.05	6.2	0.5
Mean	18.6	0.8 (96%)	18.8	1.2 (92%)

Values in parentheses indicate the mean percentage reduction in allergen concentrations from the two different methods of extraction of dust samples after treatment with tannic acid.

TABLE III. Effect of protein (BSA) in the extraction buffer on *Fel d* I concentrations in dust treated with 3% TA in the laboratory

	Method of extraction			
	BBS		5% BSA PBS	
Sample	Control	TA	Control	TA
Fel d I content (μg/gm):				
C.V.*	60.8	16	149	48.4
B.C.*	66.4	20.3	78.2	14.5
H.J.*	151	11.7	141	42.2
S.M.*	141	3.8	130	17
U.N.*	145	2.4	166	10.4
L.T.	774	2110	925	2050
M.G.	1480	1760	1870	2500

*For the samples containing <200 μg *Fel d* I/gm the mean percent decrease after TA treatment was 80% when extracted in 5% BSA PBS, but 86% when extracted in BBS.

in samples where pretreatment levels were highest, with reductions ranging from 91% to 96% for allergen levels >12 μg/gm (data not shown). TA showed marked denaturing effects on cat allergen (80% mean reduction in *Fel d* I), but only in samples containing <200 μg *Fel d* I/gm (Table III). More detailed studies in the laboratory showed that a concentration of 1% TA was required to cause a >50% reduction in allergen levels in house dust, with the exception of *Der f* I, which was more susceptible to denaturation by TA (Table IV). The lack of a significant effect of TA on dust samples with very high levels of *Fel d* I suggested that the allergen itself may act to block TA. To test this hypothesis, we added extracts of dust samples with high levels of *Fel d* I (but no detectable mite allergen) to an equal volume (250 μl) of 3% TA and incubated this mixture for 1 hour. Dust samples from two houses with significant *Der p* I content were then treated with the TA mixture and incubated for 4 hours before being extracted in 5% BSA PBS-T. The results confirmed that the presence of *Fel d* I inhibited the effect of TA on *Der p* I (Table V).

TABLE IV. Reduction of allergen levels after treatment of house dust with different concentrations of TA in the laboratory*

Concentration of TA (%)	*Der p* I (μg/gm)	% Decrease	*Der f* I (μg/gm)	% Decrease	Group II dust mite (μg/gm)	% Decrease	*Fel d* I (μg/gm)	% Decrease	Mean % decrease
0.01	30.6	<1	15.1	9	13.9	+2	117	22	8
0.05	25.7	17	13.1	22	11.7	14	124	17	17
0.1	21.3	31	11.2	33	11.2	18	91	39	30
0.2	23.2	25	8.2	51	11.4	16	93.9	37	32
1	6.7	78	1.7	90	3.4	75	60.5	60	76
3	3.7	88	0.6	96	1.7	88	29.2	81	88
Pretreatment allergen level (μg/gm)	30.8		16.7		13.6		149.6		

*0.1 gm house dust treated with 250 μl TA and incubated for 4 hours before extraction in 2 ml 5% BSA PBS-T and assay by ELISA. Results expressed are the mean of three house dust samples.

TABLE V. Effect of 3% TA on *Der p* I content of dust samples from two houses after treatment of sample in the laboratory in the presence of different concentrations of cat extract

Concentration of *Fel d* I mixed with TA (μg/ml)*	*Der p* I Concentration (μg/gm)	
	House A	House B
0†	5.3	4.4
31	10.2	2.8
46	17.2	5.3
61	14.1	9.7
92	20.2	14.1
123	22.7	15.3
Pretreatment	46.1	13.5

*Equal volumes of cat extract mixed with TA and incubated at room temperature for 1 hour before adding to dust samples from houses A and B and incubating for a further 4 hours.
†Deionized water mixed with TA.

Finally, one dust sample with a high concentration of *Der f* I and another with high *Der p* I content were treated with 3% TA for different periods, ranging from 1 minute to 6 hours, and then extracted in either BBS or 5% BSA PBS-T. Extracts of both dust samples prepared in the absence of protein showed an apparent abolition of *Der f* I and *Der p* I in 1 minute (Fig. 2). However, when samples were extracted in the presence of BSA it became clear that denaturation was a relatively slow process, taking up to 4 hours for completion.

DISCUSSION

This study demonstrated that TA interfered with the ELISA control curves for mite, cat, and cockroach allergens at concentrations as low as 0.1%. TA did not act by virtue of its pH or by a direct effect on the coating mAb or allergenic extract. One possible mode of its action was interference with binding of the allergen to the coating mAb. When dust collected from a treated carpet is extracted in saline solution, residual TA (presumably in the form of dried flakes) is redissolved and made available both to inhibit the ELISA and to further denature allergen in the extract, though the latter effect is probably not significant. Such effects could result in spuriously low allergen levels. The addition of protein (albumin) to the buffer used for extraction of dust blocked the inhibitory effect of TA on the ELISA. Maximal blocking of the effects of TA occurred when a 5% BSA (i.e., 5 gm/100 ml) saline buffer was used for extraction of dust. After extraction of dust from treated carpets, in the presence of BSA, as opposed to in saline solution alone, we

observed significant reductions in allergen in most houses, resulting in a mean reduction of >30% and >75% reduction for either *Der p* I or *Der f* I or both allergens in three houses (data not shown).

Experiments conducted in the laboratory suggested that TA was effective at reducing the antigenicity of both group I and group II mite allergens. The use of 5% BSA to extract dust significantly altered the results for group I, but had little effect on group II results. We believe that these results reflect the true susceptibility of these allergens to TA. *Der p* I and *Der f* I levels were decreased by a mean of 74% and 92%, respectively, after treatment with 3% TA when dust samples were extracted in the presence of BSA and assayed by ELISA. Under similar conditions, group II allergens were reduced by a mean of 71%. The largest reduction in mite allergen levels was seen for dust samples in which the pretreatment allergen levels were highest. A concentration of 1% TA was required to reduce allergen levels by >50%, whereas a 3% TA solution resulted in a mean decrease for all allergen levels of 88%. These results are in contrast to a recent study by Thompson et al.,[33] in which a minimum concentration of 0.2% to 0.6% TA had a maximum denaturing effect on *Der p* I in vitro. In addition, that study revealed a direct correlation between the abolition of immunochemical activity of *Der p* I and mite enzyme activity after treatment. Using BSA in the extraction buffer also allowed us to study the time course of denaturation in the laboratory. Surprisingly, 3% TA continued to denature *Der f* I and *Der p* I for up to 4 hours after mixing with house dust samples, even though approximately 60% of the allergen was denatured rapidly.

These data suggest that previous studies incorporating the ELISA method for the evaluation of TA may have overestimated its effects on dust mite allergen. The results support the use of TA as a denaturing agent for mite allergens, but demonstrate that precautions must be taken in evaluating allergen levels in dust samples obtained from treated carpets or furniture.

Preliminary results had suggested that TA would denature cat allergen, *Fel d* I.[34] In these studies effective reductions were achieved on dust samples that contained <200 μg *Fel d* I/gm, but TA had little or no effect on samples with >750 μg *Fel d* I/gm. This implied that *Fel d* I itself (or another cat-derived protein) could act to block the effect of TA. Thus the presence of one protein in sufficient quantities may impede the protein-denaturing action of TA, both on itself or on another allergen in the same sample. This is potentially more of a problem with cat allergen than with mite allergen because it is relatively uncommon to encounter mite allergen levels >200 μg/gm house dust. The results also raised the possibility that other nondefined proteins in the dust could influence the effects of TA either on allergens in the dust at the time of treatment or on the assay. Variations in the protein content of dust samples from different houses may explain why the addition of 5% BSA to the extraction buffer had different effects on assay results for different dust samples.

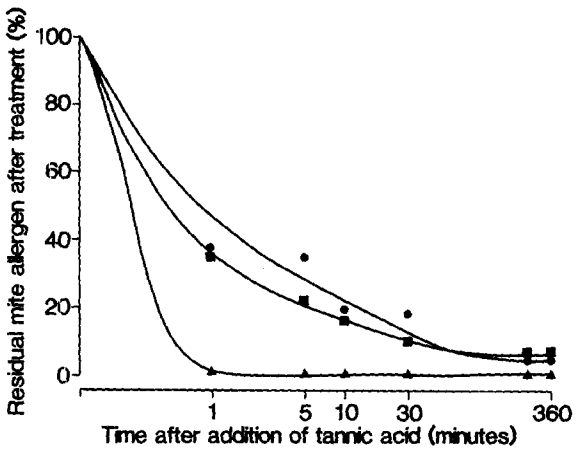

FIG. 2. Time course of action of 3% TA on dust mite allergens in two dust samples—sample A, with 54 μg/gm *Der p* I, and sample B, with 40 μg/gm *Der f* I. *(Triangles)* Mean data for residual *Der p* I and *Der f* I in both samples after extraction in BBS. Also shown are levels of residual *Der p* I in sample A *(squares)* and residual *Der f* I in sample B *(circles)* after treatment with TA and extraction in 5% BSA PBS-T.

Our results confirmed that TA was very effective at reducing mite allergen levels in dust, though it required up to 4 hours to exert its full effect. Our data show that a ≥1% TA solution should be considered an effective means of reducing mite allergen and low concentrations of cat allergen when incorporated into an allergen-avoidance regimen. Aggressive vacuum cleaning to reduce the total protein content of carpets or upholstered furnishings should be carried out before treating with TA to obtain optimal results, particularly for cat allergen. The addition of adequate protein to the buffer used for extraction of treated dust may effectively block any effects of TA on the ELISA.

We are grateful to Allergy Control Products (Ridgefield, Conn.) for providing the carpet treatments and thank Madeleine Watkins for preparing the figures and Nancy Malone for preparing the manuscript.

REFERENCES

1. Bernton HS, Brown H. Insect allergy—preliminary studies of the cockroach. J ALLERGY CLIN IMMUNOL 1964;35:506-513.
2. Voorhorst R, Spieksma FThM, Varekamp H, Leupen MJ, Lyklema AW. The house dust mite *(Dermatophagoides pteronyssinus)* and the allergens it produces: identity with the house dust allergen. J Allergy 1967;39:325-39.
3. Voorhorst P, Spieksma FThM, Varekamp N. House dust mite atopy and the house dust mite *Dermatophagoides pteronyssinus* (Troussart, 1897). Leiden: Stafleu's Scientific Publishing, 1969.
4. Ohman JL, Lowell FC, Bloch KJ. Allergens of mammalian origin, III: properties of a major feline allergen. J Immunol 1974;113:1668-77.
5. Ohman JL, Kendall S, Lowell FC. IgE antibody to cat allergens in an allergic population. J ALLERGY CLIN IMMUNOL 1977;60:317-23.
6. Sarsfield JK, Gowland G, Toy R, Norman AL. Mite sensitive asthma of childhood: trial of avoidance measures. Arch Dis Child 1974;49:711-15.
7. Walshaw MJ, Evans CC. Allergen avoidance in house dust mite sensitive adult asthma. Q J Med 1986;58:199-215.
8. Platts-Mills TAE, Tovey ER, Mitchell EB, Moszoro H, Nock P, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. Lancet 1982;2:675-8.
9. Murray AB, Ferguson AC. Dust-free bedrooms in the treatment of asthmatic children with house dust or house dust mite allergy: a controlled trial. Pediatrics 1983;71:418-22.
10. Dorward AJ, Colloff MJ, Mackay NS, McSharry C, Thomson NC. Effect of house dust mite avoidance measures on adult atopic asthma. Thorax 1988;43:98-105.
11. Platts-Mills TAE, Chapman MD. Dust mites: immunology, allergic diseases, and environmental control. J ALLERGY CLIN IMMUNOL 1987;80:755-79. (Includes a review of early studies on allergen avoidance).
12. DeBlay F, Chapman MD, Platts-Mills TAE. Airborne cat allergen (*Fel d* I): environmental control with the cat *in situ*. Am Rev Respir Dis 1991;143:1334-9.
13. Ehnert B, Lau-Schadendorf S, Weber A, Buettner P, Schou C, Wahn U. Reducing domestic exposure to dust mite allergen reduces bronchial hyperreactivity in sensitive children with asthma. J ALLERGY CLIN IMMUNOL 1992;90:135-8.
14. Colloff MJ. Use of liquid nitrogen in the control of house dust mite populations. Clin Allergy 1986;16:41-7.
15. Heller-Haupt A, Busvine JR. Tests of acaricides against house dust mites. J Med Entomol 1974;11:551-8.
16. Saint-Georges-Gridelet D, Kniest FH, Schober G, Penaud A, van Bronswijk JEMH. Lutte chimique contre les acariens de la poussiere de maison. Notes Preliminaire Rev Franc Allergol 1988;28:131-8.
17. Mitchell EB, Wilkins S, Deighton JM, Platts-Mills TAE. Reduction of house dust mite allergen levels in the home: use of the acaricide, primiphos methyl. Clin Allergy 1985;15:235-40.
18. Penaud A, Nourrit J, Autran P, Timon-David P, Jacquet-Francillon M, Charpin J. Methods for destroying house dust pyroglyphid mites. Clin Allergy 1975;5:109-14.
19. Hayden ML, Rose G, Diduch KB, et al. Benzyl benzoate moist powder: investigation of acaricidal activity in cultures and reduction of dust mite allergens in carpets. J ALLERGY CLIN IMMUNOL 1992;89:536-45.
20. Green WF, Nicholas NR, Salome CM, Woolcock AJ. Reduction of house dust mites and mite allergens: effects of spraying carpets and blankets with Allersearch DMS, an acaricide combined with an allergen reducing agent. Clin Exp Allergy 1989;19:203-7.
21. Longstaff MA, McNab JM. The effect of concentration of tannin-rich bean hulls (Vicia faba L.) on activities of lipase (EC.3.1.1.3.) and alpha-amylase (EC.3.2.1.1.) indigesta and pancreas and on the digestion of lipid and starch by young chicks. Br J Nutr 1991;66(1):139-47.
22. Makkar HP, Singh B, Dawra RK. Effect of tannin-rich leaves of oak (Quercus incana) on various microbial enzyme activities of the bovine rumen. Br J Nutr 1988;60(2):287-96.
23. Horigome T, Kumar R, Okamaro K. Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes *in vitro* and in the intestine of rats. Br J Nutr 1988;60(2):275-85.
24. Green WF. Abolition of allergens by tannic acid [Letter]. Lancet 1984;2:160.
25. Tovey ER, Marks GB, Matthews M, Green WF, Woolcock A. Changes in mite allergen *Der p* I in house dust following spraying with a tannic acid/acaricide solution. Clin Exp Immunol 1991;22:67-74.
26. Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantitation of the major *Dermatophagoides* spp. allergens, *Der p* I and *Der f* I. J Immunol Methods 1989;118:227-35.
27. Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TAE. Monoclonal antibodies to the major feline allergen *Fel d* I, II: single step affinity purification of *Fel d* I, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess *Fel d* I exposure. J Immunol 1988;140:812-18.
28. Pollart S, Smith TF, Morris EC, Gelber LE, Platts-Mills TAE, Chapman MD. Environmental exposure to cockroach allergens: analysis with monoclonal antibody–based enzyme immunoassays. J ALLERGY CLIN IMMUNOL 1991;87:505-10.
29. Ovsyannikova I, Vailes L, Chapman MD. Repertoire of B cell epitopes on group II mite allergens defined by monoclonal antibodies (mAb) [Abstract]. J ALLERGY CLIN IMMUNOL 1992;89:217.
30. Hart BJ, Guerin B, Nolard N. *In vitro* evaluation of acaricidal and fungicidal activity of the house dust mite acaricide, Allerbiocid. Clin Exp Allergy 1992;22:923-8.
31. Luczynska CM, Li Y, Chapman MD, Platts-Mills TAE. Airborne concentrations and particle size distribution of allergen derived from domestic cats *(Felis domesticus):* measurements using cascade impactor, liquid impinger and a two site monoclonal antibody assay for *Fel d* I. Am Rev Respir Dis 1990;141:361-7.
32. May KR. The cascade impactor: an instrument for sampling coarse aerosols. J Sci Instru 1945;22:187-95.
33. Thompson PJ, Stewart GA, Bird CH, Miller A, Miller JD, Kıska KD. Tannic acid, an *in vitro* and *in situ* assessment of its anti-house dust mite allergen activity [Abstract]. J ALLERGY CLIN IMMUNOL 1993;91:251.
34. Miller JD, Miller A, Kaminski K, Gelber L, Chapman MD, Platts-Mills TAE. Effect of tanic acid spray on cat allergen levels in carpets [Abstract]. J ALLERGY CLIN IMMUNOL 1990;85:226.