

The magnitude of the occult prescribing of triamcinolone is unknown. Unfortunately, the patients are unaware that ambophyllin contains a steroid. Since the patients derived some amelioration of their chronic illness from ambophyllin, their belief in what they consider a "wonder drug" is understandable. It is difficult to dissuade these patients from continued use of ambophyllin without proof that it is a corticosteroid. These patients are susceptible to Addisonian crises during medical or surgical stress. There is no historical data to inform the physician that a patient receiving ambophyllin is taking triamcinolone. Hopefully, this article will alert physicians that the proprietary, office-dispensed drug, ambophyllin contains triamcinolone.

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Proteinases in extracts of the storage mite, *Aleuroglyphus ovatus*

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Storage mites are sources of allergens associated with allergic rhinitis and asthma by skin test, RAST, and nasal and bronchial challenges.¹ Storage-mite species, such as *Glycyphagus*, *Acarus*, and *Tyrophagus*, contain proteinases, esterases, lipase, glycosidases, cellulase, amylase, lysozyme, and chitinase.² *Der p* I and *Der f* I, the major allergens of the house dust mites, *Dermatophagoides pteronyssinus* (*Dp*) and *D. farinae* (*Df*), have been identified as cysteine proteinases.³ Trypsin-like serine proteinases, some of which are allergenic, are also contained in house dust mite extracts.^{2, 4}

This study investigated the proteinase activity in extracts of the storage mite, *Ao*, which is commonly

Abbreviations used

Ao: *Aleuroglyphus ovatus*
AoB: *Aleuroglyphus ovatus* body
SCM: Spent culture medium
UCM: Unspent culture medium
OD: Optical density
E-64: Trans-epoxysuccinyl-L-leucylamido-(4-guanidino)-butane

found in farming environments. Positive skin tests and RASTs to *Ao* have been demonstrated in house dust-allergic individuals in the United States.⁵

Ao was cultured in glass containers at 75% relative humidity on autoclaved rodent chow. Cultures were sieved through a 125 μ m screen to obtain a feces-rich filtrate. *Ao* was then cultured on this filtrate to exhaustion. After 2 months, the culture was sieved through a 75 μ m screen to obtain the SCM. The SCM and the UCM were defatted and air-dried, homogenized, and extracted for 20 minutes in Tris HCl, 0.1

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TABLE I. Cysteine and serine proteinase-inhibition assays and RAST results

Extract	MW (kd)	Proteolysis	E-64% Inhib	TI% Inhib	RAST*
AoB	NS	0.059	17 [†]	0	22.7
SCM	NS	0.282	15.4 [†]	96 [†]	24
Fraction					
A	<10	—	—	—	10.6
B	>10<30	0.145	17.3 [†]	36.7 [†]	22.4
C	>30	0.266	2.5 [‡]	97.5 [†]	20.6

Inhib, Inhibition; *NS*, not specified.

Extract source, MWs, and proteolysis of azocoll expressed as OD of supernatants measured at 520 nm; reactions stopped at 30 minutes (complete hydrolysis of 1 mg/ml of azocoll; OD, ~0.6); percent inhibition of proteolysis after adding E-64; percentage inhibition after adding TI; RAST results to the different extracts (nonatopic control sera, <0.37).

*Expressed as percent of the total counts added.

[†]Significant at $p < 0.01$.

[‡]Not significant.

mol/L buffer, pH 7.4. After centrifugation at 15,000 *g* for 15 minutes, the supernatants were dialyzed in distilled water with a 1500 MW cutoff dialysis membrane. *Ao* whole bodies (*AoB*) were removed from culture with a modified Tullgren apparatus. Extracts were prepared from unhomogenized *AoB* using the same methods as above. All extracts were preserved at -20°C until the assays were performed.

The SCM extract was subfractionated with 10 and 30 kd MW cutoff Centricon ultrafilters (Polysciences, Inc., Warrington, Pa.). Extract samples were loaded onto each 30 kd filter unit and centrifuged at 1000 *g* for 1 hour. The <30 kd fraction was loaded onto the 10 kd filter unit and centrifuged in the same way. The three fractions (A, <10 kd; B, >10 kd but <30 kd; and C, >30 kd) were collected and preserved at -20°C until the assays were performed.

Protein concentrations of the extracts were determined with the bicinchoninic acid method (Pierce Laboratories, Rockford, Ill.). The SCM extract contained 2.45 mg/ml; fraction A, 0.36 mg/ml; fraction B, 1.5 mg/ml; fraction C, 4.55 mg/ml; *AoB*, 1 mg/ml; and the UCM, 1.65 mg/ml.

Proteinase activity was investigated with azocoll (Sigma Chemical Co., St. Louis, Mo.) as the substrate after previously described methods.^{6,7} All reactions were stopped at 30 minutes because OD values began to plateau at that time. Cysteine proteinase-inhibition assays were performed with the addition of 100 μl of 0.01 mol/L of E-64 (Sigma Chemical Co.) in assay buffer or an equal volume of assay buffer alone to the sample and control reaction tubes immediately before the addition of azocoll. Serine proteinase-inhibition assays were performed with the addition of 500 μg of chicken egg white trypsin inhibitor type II-O (Sigma Chemical Co.) in 100 μl of assay buffer or

an equal volume of assay buffer alone to the sample and control reaction tubes just before the addition of azocoll. The RAST was performed to all fraction, SCM, and *AoB* extracts with plastic microtiter plates and a serum pool of *Ao*-allergic individuals.⁵

This study documents the presence of proteinases in *Ao*-derived extracts. Proteinase activity was observed in the SCM, *AoB*, and fraction B and C extracts, but not in the UCM and fraction A extracts. The results of cysteine and serine proteinase-inhibition assays and RAST data are presented in Table I. Proteolysis of azocoll substrate by the SCM and fraction B extracts was significantly inhibited by compound E-64. The *AoB* extract contained less proteinase activity than the SCM extract, but statistically significant inhibition of E-64 was also observed. Because of the specificity of this inhibitor, these findings confirm the presence of cysteine proteinases, at least chemically similar, to *Der p* I and *Der f* I in the SCM, fraction B, and *AoB* extracts. Assays with trypsin inhibitor demonstrated practically complete inhibition of proteolysis by the fraction C and SCM extracts, with less inhibition of the fraction B extract, and no inhibition of the *AoB* extract. The proteolytic activity observed after cysteine and serine proteinase inhibition suggests the presence of other proteinases in fraction B.

This study is similar to that of Stewart et al.² who demonstrated the proteinase activity of *Der p* I and reported on cysteine and serine proteinases and other enzymes derived from *Dp* whole body, spent-growth media, and house dust extracts.² Ino et al.⁴ investigated the proteinase activity of *Df* body extract fractionated by gel filtration, finding a 60 kd peak with trypsin-like serine proteinase activity and 20 and 30 kd peaks with cysteine proteinase activity.⁴ This study

indicates that extracts derived from the storage mite *Ao* contain enzymes that are similar to allergenic enzymes derived from the house dust mites.

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Increased concentrations of airborne grass allergen during lawn mowing

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Many individuals with perennial or seasonal rhinoconjunctivitis complain of increased symptoms when they are exposed to freshly cut grass and lawn mowing. Previous investigators have found that the history of increased nasal and ocular symptoms occurring with lawn mowing is significantly associated with positive skin test sensitivity and elevated specific IgE antibodies to grass pollens.¹ This study was undertaken to determine if airborne grass allergen is increased during lawn mowing.

MATERIAL AND METHODS

Air sampling

Volumetric air sampling was performed at two separate sites in metropolitan Detroit during a 5-week period at the beginning of the grass-pollen season. A sample was collected before, during, and after lawn mowing with a volumetric air sampler (Quantec Air, Rochester, Minn.) onto a 9 by 9 cm polytetrafluoroethylene membrane filter (Quantec Air). The air sampler was 2 feet off the ground and located near the center of the lawn. Air was sampled at 8.5

m³/hr. The lawn mowers were gas-powered, rotary, mowers without attached grass collection bags or mulching capabilities. The air temperature and wind direction and speed at street level were recorded for each collection date. Samples were collected on days when rain had not occurred during the previous 24 hours.

Elution of samples

The collected air samples were eluted in 0.5 ml of 1% bovine serum albumin/phosphate-buffered saline, pH 7.4, and 0.002% Tween 20 for 24 hours. The filter paper and debris were separated from the supernatant with a standard serum filter (Labcraft, Houston, Texas; 16 by 9 mm).

ELISA

Grass content in the samples was determined by a biotin-avidin amplified ELISA, as previously described.² Polyclonal rabbit antigass antisera was prepared by immunization of rabbits with an equal part mixture of grass-pollen extract (grass mix [*Phleum pratense*, *Dactylis glomerata*, *Lolium spp*, and *Poa pratensis*], Hollister-Stier Laboratories, Spokane, Wash. 1:20 wt/vol, 50% glycerine) in Freund's adjuvant with standard immunization technique. The rabbit antigass antisera was diluted to 1:1000 in a 60 mmol/L carbonate/bicarbonate, pH 9.2, buffer and adsorbed to plastic microwells overnight at room temperature. The coated wells were then blocked with 0.1% Tween and 1.0% bovine serum albumin/phosphate-buffered saline. Sequentially, with interspersed washes, a study sample, biotinylated rabbit antigass sera, avidin labeled with alkaline-

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