

- and between vespid and paper wasp venoms. *Ann Allergy* 1981;46:304.
12. Stafford CT, Moffitt JE, Bunker-Soler A, Hoffman DR. Comparison of in vivo and in vitro tests in the diagnosis of imported fire ant (IFA) allergy [Abstract]. *J ALLERGY CLIN IMMUNOL* 1987;79:233.
 13. Hoffman DR. Allergens in *Hymenoptera* venom. XVI. Studies of the structures and cross-reactivities of vespid venom phospholipases. *J ALLERGY CLIN IMMUNOL* 1986;78:337.
 14. Golden DBK, Valentine MD, Kagey-Sobotka A, Lichtenstein LM. Prevalence of *Hymenoptera* venom allergy [Abstract]. *J ALLERGY CLIN IMMUNOL* 1982;69:124.
 15. Golden DBK. Diagnosis and prevalence of stinging insect allergy. *Clin Rev Allergy* 1987;5:119-36.

Immunoblotting of pollen proteins: Tween 20 mediates nonspecific binding of gammaglobulins to immunoblots of oak-pollen proteins

Richard C. Loria, MD, Jason O. Prange, and H. James Wedner, MD
St. Louis, Mo.

Oak-pollen extracts, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred onto nitrocellulose, demonstrated extensive banding in the absence of human sera or with nonatopic sera with both a horseradish peroxidase-conjugated goat antihuman IgE and ¹²⁵I-labeled rabbit antihuman IgE. When the nonionic detergent Tween 20 was removed from all incubations and washes, negative banding appeared, corresponding to bands that were clearly visible when Tween 20 had been used as a blocking agent. When Tween 20 was reintroduced, the bands reappeared. These results demonstrate that, although Tween 20 is effective in blocking unbound sites on nitrocellulose membranes, this and other nonionic detergents may cause nonspecific binding to immunoblots of oak-pollen proteins. Thus, care must be used in the interpretation of immunoblots to detect allergens when Tween 20 is used as the blocking agent. (J ALLERGY CLIN IMMUNOL 1988;82:834-41.)

Since its original description by Towbin et al. in 1979,¹ the immunologic detection of proteins separated by electrophoresis and immobilized on NC paper, "immunoblotting," has become a major biologic research tool. Many investigators have already applied this technique to the analysis of complex allergenic mixtures.²⁻¹⁴ The data obtained from immunoblots of allergenic extracts will most likely be used to determine which individual proteins must be present in an allergenic extract used for the diagnosis and treatment

Abbreviations used

SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
NC:	Nitrocellulose
HRP:	Horseradish peroxidase
BSA:	Bovine serum albumin
DAB:	3,3'-diaminobenzidine
TBS:	Tris-buffered saline
4CN:	4-chloro-1-naphthol
PBS:	Phosphate-buffered saline

From the Washington University School of Medicine, Department of Medicine, Division of Allergy and Immunology, St. Louis, Mo.

Received for publication Nov. 10, 1987.

Accepted for publication April 30, 1988.

Reprint requests: H. James Wedner, MD, Washington University School of Medicine, Department of Medicine, Division of Allergy and Immunology, Box 8122, 660 S. Euclid, St. Louis, MO 63110.

of allergic individuals. Thus, conditions leading to nonspecific binding of probes, such as enzyme or radiolabeled antihuman IgE to electroblotted pollen proteins, would lead to erroneous conclusions as to the significance of these proteins in eliciting a specific antibody response.

In the process of analyzing oak-pollen extracts with

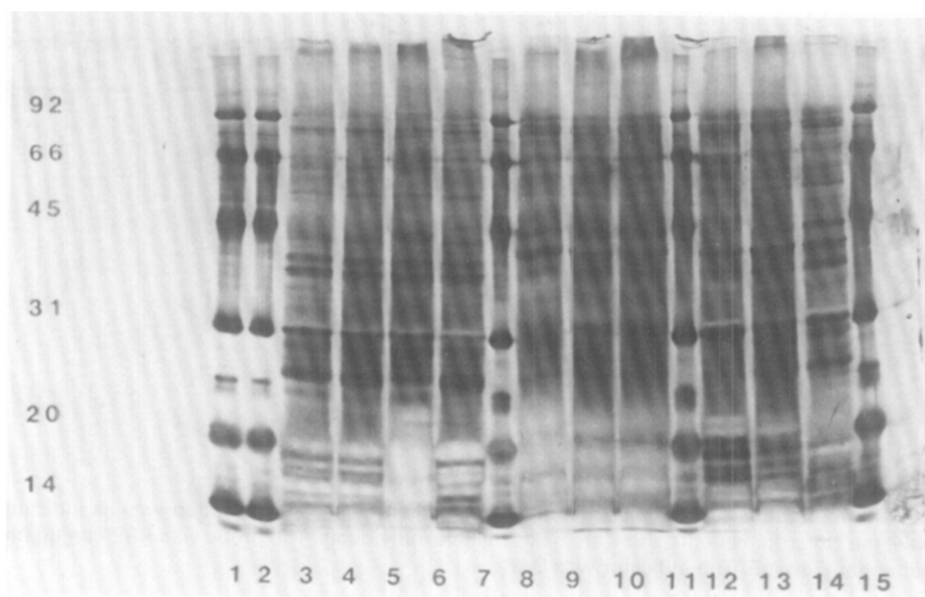


FIG. 1. SDS-PAGE of several oak-pollen species. Lanes 1, 2, 7, 11, and 15 correspond to molecular weight markers. Lanes 3 to 6 correspond to southern red oak (*Quercus falcata*) from four different suppliers. Lanes 8 to 10 correspond to gambel oak (*Quercus gambelli*). Lanes 12 to 14 correspond to western white oak (*Quercus garryana*).

immunoblotting, we noted nonspecific binding of our enzyme and radiolabeled probes to the electroblotted oak-pollen proteins. Assessment of the technical conditions of our assay pointed to a specific interaction between oak-pollen proteins and the nonionic detergents that are commonly used to block "unbound" NC membranes. It appears that this interaction leads to the binding of enzyme or radiolabeled probes to the oak-pollen proteins independent of whether or not human sera is included in the assay.

MATERIAL AND METHODS

Quantities of dry oak pollen were obtained from several suppliers: Biopol Laboratory Inc., Spokane, Wash.; Greer Laboratories, Inc., Lenoir, N.C.; Hollister-Stier Laboratories, Spokane, Wash.; Crystal Laboratory, Luther, Okla.; Ashland Farm Botanicals, Sedalia, Mo.; and International Biologicals, Piedmont, Okla. Acrylamide was from Boehringer Mannheim Biologicals, Indianapolis, Ind; *N,N'*-methylene-bis-acrylamide, ammonium persulfate, TEMED, amido black, and Tween 20 were obtained from Bio-Rad Laboratories, Richmond, Calif. Tween 20 was also obtained from Sigma Chemical Co., St. Louis, Mo., and highly purified Tween 20 (Surfact-Amps 20) was from Pierce Chemical Co., Rockford, Ill. Blocking agents were casein hydrolysate (Sigma Chemical Co.), BSA, fraction V, Calbiochem, San Diego, Calif., *N*-lauroylsarcosine and thimerosal, Sigma Chemical Co., and fish gelatin, hipure liquid gelatin, Norland Products, Inc., New Brunswick, N.J. Oak-pollen extract for skin testing was from Hollister-Stier, ¹²⁵I-labeled rabbit antihuman IgE (Phadebas IgE

PRIST) from Pharmacia Diagnostics, Fairfield, N.J., HRP-conjugated goat antihuman IgE supplied by Miles Scientific Div., Naperville, Ill., and 4CN or DAB was obtained from Sigma Chemical Co.

Oak-pollen extracts

Aqueous extracts (10% wt/vol) of various oak pollens were prepared by rotating dry oak pollen with distilled deionized water (Milli-Q, Waters Associates, Millipore Corp., Milford, Mass.) for 48 hours at 4° C. Extracts were clarified by centrifugation at 1500 g for 15 minutes on a Beckman J-6 (Beckman Instruments, Inc., Irvine, Calif.) centrifuge, and at 17000 g for 30 minutes on a Sorvall (Sorvall Instruments, Wilmington, Del.) RC-5B refrigerated superspeed centrifuge. The pollen extract was decanted off and filtered through a 0.22 micron syringe filter (Nalge; Nalge Co., Rochester, N.Y.) and then stored at 4° C. Protein concentration was determined according to the method of Lowry et al.¹⁵

SDS-PAGE

The extracts of oak pollen were separated on 15% single-well SDS-PAGE "minigels" with the buffer system described by Laemmli,¹⁶ and 200 µl of sample containing approximately 200 µg of total protein were separated at 22 mA per gel until the dye front reached the bottom of the gel. The gels were stained with either Coomassie brilliant blue (Bio-Rad Laboratories) or fixed in trichloroacetic acid/sulfosalicylic acid as described by Guevara et al.¹⁷ and stained with a silver stain (Rapid-Ag-Stain, ICN Radiochemicals, Irvine, Calif.) as described in the package insert. Gels used for immunoblotting were left unstained.

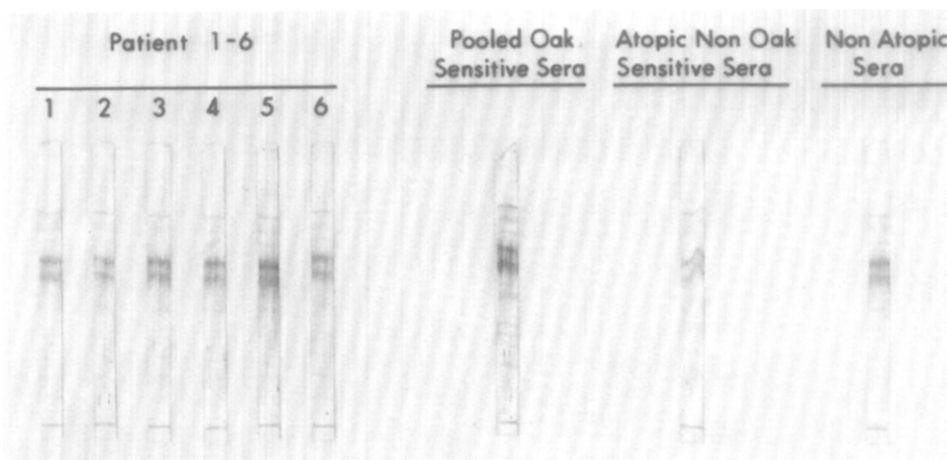


FIG. 2. Immunoblots of red oak (*Quercus rubra*) pollen. The first incubation was as indicated above the NC strip. Patients 1 to 6 are oak-sensitive individuals. The probe was HRP-conjugated goat antihuman IgE. The indicator was DAB.

Electrophoretic transfer of SDS-PAGE oak-pollen extracts onto NC

SDS-polyacrylamide gels were equilibrated in Tris/glycine/methanol buffer, pH 8.3, as described previously.¹ The gels were then placed into contact with 0.22 micron NC membrane (Bio-Rad Laboratories) and sandwiched between several layers of Whatman No. 1 (Whatman, Inc., Clifton, N.J.) filter paper and fiber pads. With a trans-blot cell (Bio-Rad Laboratories), electrophoretic transfer onto NC was performed at 90 V for 2 hours in Tris/glycine/methanol buffer. After drying the NC membrane, a small strip was removed and stained with amido black stain to confirm transfer of protein. The gels were also stained with either Coomassie brilliant blue or the silver stain to assure that all of the bands had transferred.

Blocking (quenching) of the NC membrane

The NC membranes were immersed in PBS containing 0.1% Tween 20 and gently rocked on an orbital shaker for 1 hour at room temperature. Alternative blocking conditions included alterations in the concentration of Tween 20 ranging from 0.05% Tween 20 in PBS to 1% Tween 20 in PBS, varying the incubation time in blocking agent from 1 to 2 hours, and the use of alternative blocking agents. The other blocking agents included casein hydrolysate, BSA, *N*-lauroylsarcosine, and fish gelatin.

First incubation

Sera were obtained from individuals who had positive skin tests to oak pollen. The NC membranes were cut into 0.5 cm wide strips and incubated with control or atopic sera that had been diluted 1:2 in PBS 0.1% Tween 20. Incubations ranged from 3 to 16 hours and were performed on an orbital shaker at room temperature. After aspirating the

human serum dilutions, the strips were washed twice for 20 minutes each wash in PBS 0.1% Tween 20. In several experiments extended washing times were tried.

Second incubation

The NC strips were incubated with an ¹²⁵I-labeled rabbit antihuman IgE at approximately 40,000 cpm per strip or an HRP-conjugated goat antihuman IgE diluted 1:1000 in PBS 0.1% Tween 20. The strips were incubated with the second probe for times ranging between 3 to 16 hours. After removal of the second probe, the strips were again washed twice with PBS 0.1% Tween 20 for 20 minutes each wash. Washing for extended periods of time was attempted in several experiments. The strips that had been incubated with the radiolabeled probe were then autoradiographed (Kodak XAR-5 film; Eastman Kodak Co., Rochester, N.Y.). Intensifying screens were used as needed. In experiments in which the HRP-conjugated probe was used, the strips were incubated with the peroxidase substrates 4CN or DAB. DAB was used for all experiments presented with the HRP-conjugated probes because it produced sharper bands with less fading than the peroxidase substrate, 4CN.

RESULTS

SDS-PAGE

Staining of the SDS-polyacrylamide gels of the different oak-pollen species revealed, for the most part, unique banding patterns for each species. In many instances the banding pattern for a given species from one supplier was indistinguishable from the banding pattern of the same species from a different supplier. On a few occasions, however, differences were noted. Fig. 1 illustrates the typical banding pattern observed on SDS-polyacrylamide gels for several species of

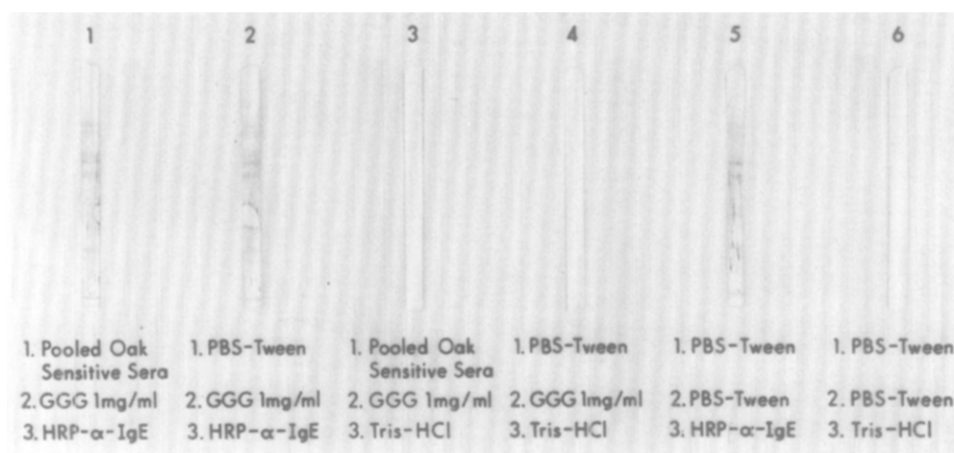


FIG. 3. Immunoblots of burr oak (*Quercus macrocarpa*) pollen. The first incubations were as noted below each NC strip by the number 1. The immunoblots were subsequently incubated with unconjugated goat gammaglobulin (GGG) or PBS-Tween, as indicated by the number 2. The probe was an HRP-conjugated goat antihuman IgE or blank (TBS) as indicated by the number 3.

oak pollen. The number of bands observed with SDS-PAGE of oak-pollen extracts is 40 to 50.

Transfer of SDS-PAGE oak extracts onto NC. As assessed by amido black staining of a strip of each NC membrane, the electrophoretic transfer to NC was very efficient. Furthermore, staining of the gels after transfer revealed that no bands remained on the SDS-polyacrylamide gels after the transfer procedure (data not presented).

Probing of oak-pollen immunoblots with an HRP-conjugated goat antihuman IgE. The immunoblotting pattern for six oak-allergic individuals is demonstrated for red oak (*Quercus rubra*) in Fig. 2. Numerous bands of red oak pollen were observed with this technique. There is no difference in the banding pattern among any of the six oak-allergic patients or the NC strip incubated with pooled sera from oak-sensitive individuals obtained from another midwestern state. The same bands developed in the peroxidase reaction even when the immunoblots of red oak were incubated with atopic sera or sera from nonoak-sensitive individuals.

Decreasing the concentration of Tween 20 to 0.05% in PBS or increasing the concentration to 1% Tween 20 did not change the results. Extending the washing times up to 1 hour with two changes of the washing solution also did not change the banding patterns. Similar results were observed regardless of the oak-pollen supplier or the species of oak pollen used.

In similar experiments with immunoblots of pin oak (*Quercus palustris*), extensive nonspecific binding of

the HRP-conjugated goat antihuman IgE to the NC bound oak-pollen proteins occurred in the absence of human sera (data not presented).

When the HRP-labeled probe was omitted from the assay, no banding was observed (Fig. 3).

Nonspecific binding of the HRP-labeled goat antihuman IgE to the oak-pollen proteins was not inhibited by incubating the immunoblots with unconjugated goat gammaglobulin after the initial incubation with sera from oak-allergic or control individuals (Fig. 3).

Probing of oak-pollen immunoblots with an ^{125} I-labeled rabbit antihuman IgE. When oak-pollen immunoblots were probed with an ^{125} I-labeled rabbit antihuman IgE, nonspecific binding also occurred (data not presented).

Exclusion of Tween 20 from the immunoblotting procedure. Based on the fact that other investigators had suggested that the nonionic detergents may mediate nonspecific binding,¹⁸⁻²⁰ we proceeded to evaluate various blocking conditions with and without Tween 20. In immunoblots in which PBS or TBS without Tween 20 were used, high levels of background staining were noted (Fig. 4). In addition, "negatively" stained bands were observed. These were best observed in immunoblots of blue oak (*Quercus douglasii*) (Fig. 4). These negatively stained bands correspond to oak-pollen proteins, as observed by amido black staining of the NC membrane. When another nonionic detergent, Triton X-100 was used, similar nonspecific binding occurred (data not presented).

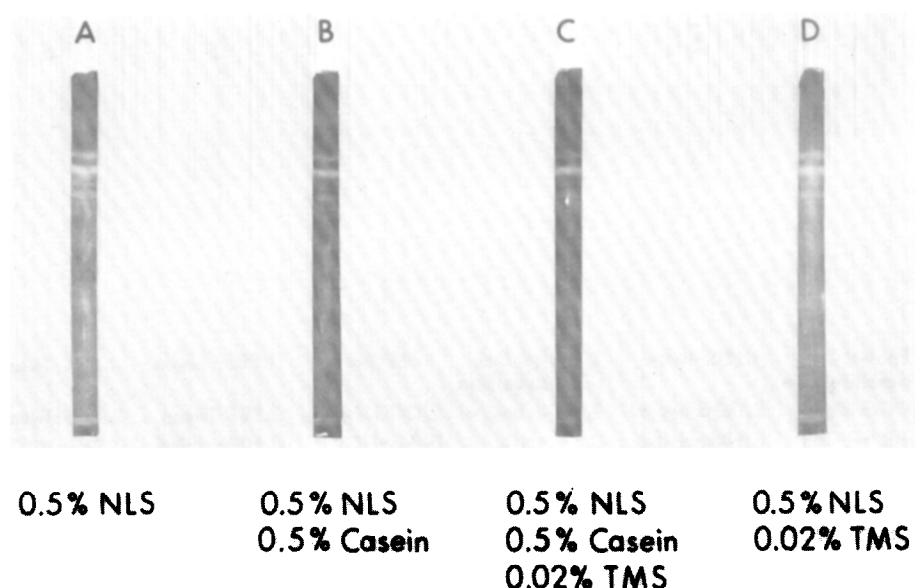


FIG. 4. Immunoblots of blue oak (*Quercus douglasii*) pollen. The first incubation was with TBS. The probe was an HRP-conjugated goat antihuman IgE. The indicator was DAB. The blocking conditions varied, as noted below each NC strip.

Use of a highly purified preparation of Tween 20. Because highly reactive oxidizing impurities had previously been found in the nonionic detergents,^{21, 22} we used a highly purified preparation of Tween 20. Although we observed a less prominent nonspecific binding pattern, it was still present.

Use of alternative blocking agents. *N*-lauroylsarcosine alone or in combination with casein and/or thimerosal proved to be ineffective blocking agents in that the background signal was very high. The pollen bands, however, were left unstained (Fig. 4). Use of BSA and gelatin led to a marked reduction in nonspecific binding, although one to two bands were still visible on each NC strip (data not presented).

DISCUSSION

Immunoblotting has established itself as a powerful tool in analytical protein chemistry. Transfer of separated mixtures of complex proteins onto NC membranes provides a solid phase whereby the components of the mixture can be reacted with appropriate dilutions of atopic or control human sera. Incubation with a second probe, in the case of allergy extracts, an enzyme or radiolabeled antihuman IgE or an enzyme or radiolabeled antihuman IgG allows visualization of the allergens. When this is performed properly, control NC strips should be incubated at the same dilutions, for the same length of time, and reacted with the same amount of probe as are strips incubated with the atopic sera. On development of the enzymatic

reaction or exposure of ¹²⁵I-labeled probes to x-ray film, control strips should be devoid of any positive signals, whereas NC strips incubated with sera from allergic individuals will demonstrate discrete bands corresponding to the allergens to which they are sensitive. Mixtures, such as silk polypeptides,² *Dermaphagoides pteronyssinus* extracts,³ grass-pollen extracts,⁴⁻⁹ tree-pollen extracts,^{10, 11} wheat-flour extracts,¹² and even venom extracts,^{13, 14} have been examined with immunoblotting. In many of these studies the authors have obtained specific immunoblotting patterns with atopic sera compared to that of control sera.

In studying the allergenic proteins in oak-pollen extracts separated by SDS-PAGE and blotted onto NC, we noted positive signals with an enzyme-labeled probe and with a radiolabeled probe on our control NC strips. Results were similar for all oak-pollen species tested, regardless of the oak-pollen supplier, and therefore excluding this phenomenon as being unique to any one species of oak or supplier handling of the pollen.

Positive signals appeared on the control NC strips whether the initial control incubation was with atopic sera, sera from nonoak-sensitive subjects, nonatopic sera, or simply PBS-Tween alone. The appearance of positive signals in the absence of human sera suggested that the labeled probe was binding to pollen proteins rather than to human immunoglobulins. Decreasing the incubation times to 3 hours did not reduce

the nonspecific binding. When enzyme-labeled probes were used, 10-minute washes are usually sufficient to minimize nonspecific reactions.²³ Even extending the washing times up to 1 hour with two changes of the washing solution did not decrease the nonspecific binding.

Both of the peroxidase substrates used, 4CN and DAB, elicited similar results. This, along with data that demonstrated that no aberrant peroxidase activity was associated with the pollen proteins, demonstrated that nonspecific binding could not be attributed to the peroxidase substrates.

Our initial experiments used an HRP-conjugated goat antihuman IgE. The coupling procedure that was used to conjugate the HRP to the goat gammaglobulin was according to the method described by Avrameas and Ternynck.²⁴ We suspected that this probe might have been altered by the conjugating procedure or that residual amounts of glutaraldehyde, the coupling reagent, might have been present in the commercial preparation. We therefore attempted to use a radio-labeled probe. The results obtained with an ¹²⁵I-labeled rabbit antihuman IgE were no different, however. Nonspecific binding therefore could not be attributed to residual coupling reagent in the enzyme-labeled preparation, alteration of the goat gammaglobulin by the coupling procedure, or a peculiarity of goat globulins.

After excluding the pollen, human sera, various probes, and substrates as causes of the nonspecific binding, we then examined several technical conditions of the assay. One of the most crucial technical aspects of the immunoblotting procedure is the blocking or quenching of the NC membrane. Omission of, or inadequate blocking, would allow binding of serum components, among these serum antibodies, to the NC membrane. Nonspecific or "nonsense" reactions would be difficult to exclude without assurance that only components of the original allergen extract were bound to and hence probed on the membrane. Important factors in the election of which blocking agent to use included the characteristics of the membrane, the type of protein mixture being analyzed, and the specific enzyme conjugate used. Along these lines, Tween 20 and several of the other nonionic detergents have been successfully used in blocking NC membranes. Successful blocking has also been achieved with BSA, hemoglobin, gelatin, horse serum, and nonfat dried milk.^{25, 26} In the event that a peroxidase-conjugated probe is used, there should be no trace of azide in any of the solutions, since this inhibits the peroxidase.²⁷ Additionally, because it may contain aberrant peroxidase activity, hemoglobin should not

be used as a blocking agent when the probing antibody is conjugated to HRP (Zeta-Probe blotting membrane instruction manual, Bio-Rad Laboratories). Ultimately, the choice of an appropriate blocking agent depends on trial and error, with inclusion of the appropriate controls.

A review of the literature reveals that many investigators that use immunoblotting in the analysis of complex allergenic mixtures have used Tween 20 as the blocking agent.^{2-5, 7, 9, 12} Nonspecific binding, however, was not described by any of these authors. In only one of these studies⁷ was another tree-pollen extract (birch, *Betula verrucosa*) examined. Nonspecific binding was carefully controlled in this study and did not occur.

Although our initial impression was that this phenomenon might be due to a particularity of oak-pollen proteins, several groups have also directly implicated the nonionic detergents as a cause of nonspecific binding in immunoassays involving nonoak-pollen proteins. Kenna et al.,¹⁸ using PBS-Tween 20 in washing and antibody diluent buffers, noted significant direct binding of rabbit IgG to micro-ELISA wells and high binding of their preimmune rabbit antibody and of second antibody (antirabbit IgG) to rabbit liver plasma membrane fraction. They also noted high direct binding of human IgG to the solid phase when PBS-Tween 20 was used in wash and antibody diluent buffers. When these authors replaced the PBS-Tween 20 with nonreactive proteins, such as casein, BSA, or gelatin, the nonspecific binding was significantly decreased.

Perini et al.¹⁹ obtained high levels of nonspecific binding with nonimmune rabbit antisera to immunoblotted human urinary proteins when nonionic detergents were included in the wash and antibody diluent buffers. One manufacturer of "Western blotting" reagents (Janssen Pharmaceutical, Piscataway, N.J.) also notes in one of their package inserts (AuroProbe and BLplus-IntenSE) that the use of Tween-20 led to nonspecific binding of gold probes to blotted proteins in certain types of samples. Finally, Gershoni and Palade,²⁰ in reviewing protein blotting, mentioned that the use of nonionic detergents has led to "false positives." They suggest that this may result from nonspecific binding of hydrophobic probes to hydrophobic domains of denatured polypeptides, possibly mediated by the nonionic detergents.

When Tween 20 was omitted from all experiments, high background staining was observed. Readily apparent against this dark background, however, were well-defined "negatively" stained bands. That only the pollen bands were left unstained when Tween 20 was

omitted indicated a specific interaction between the pollen and the Tween 20. Whether the nonionic detergents are actually bridging hydrophobic probes to hydrophobic domains of denatured polypeptide, as suggested by Gershoni and Palade,²⁰ is unknown. The occurrence of nonspecific binding in the presence of Tween 20 does, however, support the notion that some antigens are "detergent sensitive,"¹⁸ and, in these cases, alternative reagents should be used. Our experiments using Triton X-100 confirmed that the problem might be generalized to include the other nonionic detergents as well.

The possibility that oxidizing impurities in the Tween 20^{21, 22} was the cause of the nonspecific binding was considered. Using a highly purified preparation of Tween 20, we noted persistent, albeit less, nonspecific binding. This appeared to support a specific interaction between the pollen proteins and the nonionic detergents.

The presence of antibodies in human serum specific for microbial contaminants could have caused nonspecific binding had these contaminants actually been bound to the NC. The results we obtained, however, were the same, regardless of whether or not human sera was included in the assay, thereby excluding this possibility.

Although the data presented are limited to several species of oak pollen, data from our laboratory* and from other investigators† indicate that the problem of nonspecific binding may also occur with pollen proteins from various species of grass.

CONCLUSION

To date, problems with regard to nonspecific binding in immunoblotting of allergenic extracts have not, to our knowledge, appeared in the literature. Our results, however, confirm a specific interaction between the nonionic detergents and oak-pollen proteins leading to nonspecific binding of labeled probes to the pollen. Although we suspected that this problem might be limited to pollen proteins, several references from the literature described nonspecific binding in assays of proteins of a very diverse nature in which a nonionic detergent was used. Because it is such a powerful tool, immunoblotting will likely be heavily relied on to both identify allergens and compare allergen extracts. Thus, application of this technique must be performed with a thorough knowledge of not only its applications but also its potential problems.

*Loria RC, Wedner J. Unpublished observations.

†Reid M. Personal communication.

REFERENCES

1. Towbin A, Staehelin T, Gordon V. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979;76:4350.
2. Dewair M, Baur X, Ziegler K. Use of immunoblot technique for detection of human IgE and IgG antibodies to individual silk proteins. *J ALLERGY CLIN IMMUNOL* 1985;76:537.
3. Tovey ER, Baldo BA. Standardization of allergens: qualitative definition of house dustmite extracts following electroblotting and detection of components with antibody and lectin probes. *Int Arch Allergy Appl Immunol* 1984;75:322.
4. Haas H, Becker WM, Maasch HJ, Schlaak M. Analysis of allergen components in grass-pollen extracts using immunoblotting. *Int Arch Allergy Appl Immunol* 1986;79:434.
5. Ford SA, Tovey ER, Baldo BA. Identification of orchard grass (*Dactylis glomerata*)-pollen allergens following electrophoretic transfer to nitrocellulose. *Int Arch Allergy Appl Immunol* 1985;78:15.
6. Ekramoddoullah AKM, Kisil FT, Sehon AH. Isolation of Kentucky bluegrass-pollen allergen using a murine monoclonal antibody immunosorbent. *Int Arch Allergy Appl Immunol* 1986;80:100.
7. Bengtsson A, Rolfsen W, Einarsson R. Characterization of allergens and patient sera by a nitrocellulose immunoprint technique. *Int Arch Allergy Appl Immunol* 1985;78:139.
8. Ekramoddoullah AKM, Kisil FT, Sehon AH. Partial characterization of an antigenic site of high molecular weight basic antigen, a ryegrass pollen allergen, using a monoclonal antibody. *Mol Immunol* 1986;23:111.
9. Ford SA, Baldo BA. Identification of Bermuda grass (*Cynodon dactylon*)-pollen allergens by electroblotting. *J ALLERGY CLIN IMMUNOL* 1987;79:711.
10. Florvaag E, Elsayed S, Hammer ASE. Comparative studies on tree-pollen allergens. XIII. Partial characterization of the Alder (*Alnus incana*)-pollen extract by two-dimensional IEF/SDS-PAGE electrophoresis combined with electrophoretic transfer and immunautoradiography. *Int Arch Allergy Appl Immunol* 1986;80:26.
11. Ito Y, Yoshinaka Y, Ohi M, Sakakura Y. Analysis by electrophoretic transfer blotting of Japanese cedar-pollen allergens which react with IgG and IgE antibodies in the serum of patients. *Int Arch Allergy Appl Immunol* 1981;1:174.
12. Theobald K, Thiel H, Kallweit C, Ulmer W, König W. Detection of proteins in wheat flour extracts that bind human IgG, IgE, and mouse monoclonal antibodies. *J ALLERGY CLIN IMMUNOL* 1986;78:470.
13. Jacobson RS, Hoffman DR. The European hornet (*Vespa Crabro*), an emerging sting problem in the eastern U.S. [Abstract]. *J ALLERGY CLIN IMMUNOL* 1985;75:121.
14. Jacobson RS, Hoffman DR. Allergens in imported fire ant venom [Abstract]. *J ALLERGY CLIN IMMUNOL* 1986;77:143.
15. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagent. *J Biol Chem* 1951;193:265.
16. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680.
17. Guevara J Jr, Johnston DA, Ramagali LS, Martin BA, Capetillo S, Rodriguez LV. Quantitative aspects of silver deposition in proteins resolved in complex polyacrylamide gels. *Electrophoresis* 1982;3:197.
18. Kenna JG, Major GN, Williams RS. Methods for reducing

- nonspecific antibody binding in enzyme-linked immunosorbent assays. *J Immunol Methods* 1985;85:409.
19. Perini JM, Dehon B, Marianne T, Klein A, Roussel P. Reducing the artifacts produced by impure antisera in immunoblots of low-molecular-mass proteins in urine. *Clin Chem* 1986;32(suppl 5):811.
 20. Gershoni JM, Palade GE. Protein blotting: principles and applications. *Anal Biochem* 1983;131:1.
 21. Chang HJ, Bock E. Pitfalls in the use of commercial nonionic detergents for the solubilization of integral membrane proteins: sulfhydryl oxidizing contaminants and their elimination. *Anal Biochem* 1980;104:112.
 22. Ashani Y, Catravas GN. Highly reactive impurities in Triton X-100 and Brij 35: partial characterization and removal. *Anal Biochem* 1980;109:55.
 23. Bers G, Garfin D. Protein and nucleic acid blotting and immunobiochemical detection. *Biotechniques* 1985;3:276.
 24. Avrameas S, Ternynck T. Peroxidase-labeled antibody and Fab fragments: conjugates with enhanced intracellular penetration. *Immunochemistry* 1971;8:1175.
 25. Gershoni JM. Protein blotting: developments and perspectives. *Trends Biochem Sci* 1985;10:103.
 26. Towbin H, Gordon J. Immunoblotting and dot immunobinding—current status and outlook. *J Immunol Methods* 1984;12:313.
 27. Walsh BJ, Wrigley CW, Baldo BA. Simultaneous detection of IgE binding to several allergens using a nitrocellulose "poly-disc." *J Immunol Methods* 1984;66:99.

Correction

The following change applies to the article "Dust mites: Immunology, allergic diseases, and environmental control" by Platts-Mills and Chapman, which appeared on page 755 of the December 1987 issue of *The Journal of Allergy and Clinical Immunology*.

Fig. 7 was not well produced in the original publication. The figure below has been properly printed.



FIG. 7. Transmission electron microscopy of a house dust sample. Three separate fecal particles are illustrated with distinct peritrophic (chitinous) membranes. This sample of dust contained $>10 \mu\text{g/gm}$ of *Der p* I. The *sizing bar* indicates $10 \mu\text{m}$. The *small size* reflects the fact that sections are not through the center of the particles.