

***In Vivo* Diagnostic Procedures: Skin Testing, Nasal Provocation, and Bronchial Provocation**

R. Stokes Peebles, Jr., and Tina V. Hartert

Division of Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

In vivo challenge procedures can be very useful in the analysis of allergic symptoms. Skin testing has a high degree of sensitivity and specificity for determining antigens that cause allergic disease. However, positive skin tests do not necessarily indicate that a specific allergen causes symptoms specific for a certain organ. Nasal and whole lung provocation testing can help define relevant allergens that cause rhinitis or asthma symptoms. These tests are safe when performed properly under close medical supervision and have predictive values that make them useful diagnostic tools. © 1997 Academic Press

To the general public, allergy is a conundrum. The fact that allergic individuals have reactions that range from almost unnoticeable to life-threatening on exposure to substances that cause no problem to the large majority of the population is both fascinating and intimidating, particularly to the person at risk. To the majority of nonimmunologically trained physicians, allergy testing is also mysterious and largely misunderstood. It is mysterious because a diagnosis may be made as simply as placing a drop of the substance in question on the skin and scratching the area where the drop was placed, a practice tantamount to trial by ordeal. Allergy testing is largely misunderstood for two main reasons. First, technologic advances in immunology research and the ability to apply this research to the diagnosis of allergic disease outpace the capability of the non-specialist to keep up with these discoveries. Second,

a few physicians and laboratories have taken advantage of the difficulty that the nonimmunologically trained specialist has in maintaining an understanding of complicated testing techniques and subsequently use diagnostic practices that have no basis in scientific fact and that are quite expensive. Therefore, it is important for the referring physician to have a basic understanding of allergy testing. The goal of this article is to provide the current knowledge of *in vivo* allergy testing techniques and accurate interpretation of the results of these tests.

Specifically, this article will cover immediate hypersensitivity and the *in vivo* methods used to detect the presence of antigen-specific immunoglobulin E (IgE) antibodies. These methods include skin testing to aeroallergens, medications, and venoms, as well as nasal and bronchial provocation techniques. Methacholine and histamine bronchial provocation testing, methods that do not determine the presence of IgE antibodies against a specific substance, but that are widely used to aid in the diagnosis of asthma will also be discussed.

The manifestations of immediate hypersensitivity can be quite varied and range from hives (urticaria) and hayfever (allergic rhinoconjunctivitis) to fatal asthma and anaphylaxis. The basis of immediate hypersensitivity is the production of IgE antibody by a patient's B-lymphocytes against a specific substance, an antigen, that the patient has encountered in his or her environment (1). The IgE antibody that recognizes and binds this particular environmental substance is termed "antigen-specific IgE antibody." IgE antibodies are made by B-lymphocytes and circulate in the body, adhering to the surface of mast

cells in various organs. Reexposure to the antigen, the substance against which the IgE antibody was specifically made, leads to an allergic reaction as the antigen cross-links antigen-specific IgE antibodies on the mast cell surface. Antigen reexposure can occur through skin contact, insect sting, inhalation into the respiratory tract, ingestion, or intravenous administration. The antigen cross-linking of IgE on the mast cell's surface results in mast cell degranulation and mast cell mediator release. Mediator release initiates a chain of events that result in the end organ manifestations of an allergic reaction (1). An antigen that leads to an allergic reaction is termed an allergen.

SKIN TESTING

Immediate hypersensitivity skin testing is performed both to determine the presence of IgE antibodies in the skin that recognize the substance being tested and to link specific antigens with allergic symptoms that may be occurring elsewhere in the body (2). The presence of a positive skin test to an antigen in a person who has rhinitis or asthma is commonly interpreted as that antigen may be capable of causing respiratory symptoms, even though the nose and lungs are not being specifically challenged. Likewise, a negative skin test is generally used to rule out an IgE-mediated process to the antigen being tested. Thus, the major indication for skin testing is to determine if a substance in the environment may be responsible for any of the symptoms associated with type I hypersensitivity reactions. The power of skin testing as an accurate marker of the presence of allergic disease to a particular antigen is enhanced by a careful environmental history and the physician's knowledge of antigens to which the patient may be exposed, but of which the patient is not aware. Thus, an approach in which a large number of antigens are tested, yet for which there is no reason to suspect the patient has possible exposure, is wasteful, both in regard to what the patient may pay, and in needless concern. Therefore, skin tests should be performed only with antigens that have a reasonable possibility of causing an individual's symptoms (2).

The two most commonly used methods of skin testing are the prick and intradermal tests. Both tests are safe when performed properly, but both have the potential to cause systemic allergic reactions and therefore should be executed only when a physician

is present and when emergency equipment is immediately available to treat a severe reaction. This equipment should include 1:1000 aqueous epinephrine, antihistamines, oxygen, and the materials needed for intravenous fluid administration. In the event that a mild systemic reaction occurs, 0.3 ml of 1:1000 aqueous epinephrine should be administered subcutaneously and antihistamines should be given orally; however, a more severe reaction should result in the same medications being given, immediate activation of the Emergency Medical Service, oxygen administration, placement of intravenous access, and transportation to the nearest emergency department capable of handling life-threatening situations (2).

Prick tests are usually performed on either the volar surface of the arms or on the back. It is important to avoid areas of wounds or eczema and perform testing only in regions of normal skin, both for reliability of test results and for patient safety. The test site is cleansed with either 2-propanol or 70% ethanol to avoid the possibility of infection. After the cleaning solution has dried, each testing site is marked with a code that will identify the antigen placed at that particular site. Adjacent skin test sites should be separated by at least 4 cm to avoid overlap of large positive reactions. A single drop of a test solution is applied at the appropriate test site from a rubber bulb dropper, with care that the dropper tip does not touch the skin, so that the remaining solution in the bottle is not contaminated by skin microorganisms when the dropper is placed back in the container. A 26-gauge disposable sterile needle is then passed through the drop of the skin test solution and inserted into the skin with the bevel of the needle facing away from the skin surface. The skin is slightly lifted and the needle is removed. The ability to lift the skin signifies that the epidermis was indeed punctured; however, the needle should not be inserted so deeply as to cause bleeding. After 1 min, the test solution is wiped away with a clean tissue paper to prevent contamination of the test solution from one site to the next. For the same reason, a new needle should be used at each site or the needle may be cleansed with saline and ethanol between punctures (2).

Control skin tests are also placed to increase the validity of the procedure. Histamine is used as a positive control, as all persons not on medications should develop a wheal and an erythema reaction to this substance. The relevance of using histamine is based on the recognition that histamine is a major mediator released by mast cells and accounts for a

large portion of the allergic response. Antihistamines are used by a large segment of the allergic population to control symptoms; however, the therapeutic effect of these medications is to block the actions of histamine on target organs, including the wheal and erythema in the skin. Thus, taking antihistamines or other medications with antihistaminic properties, such as tricyclic antidepressants, may give false-negative results and make skin testing invalid. To assess the possibility that a medication may interfere with skin test results, a 1.8 mg/ml histamine solution is used as a control prick test. If a subject has a negative histamine control, it is necessary to wait until the effects of the medication have disappeared and the appropriate histamine response is noted or antigen-specific IgE can be measured in serum by RAST (radioallorbsorbent test). It is important to note that systemic, inhaled, or intranasal corticosteroids, also used to treat allergic symptoms, do not generally interfere with skin test results. Saline is used as a negative control to assess whether the subject has dermographism, a condition that produces nonspecific false-positive skin tests. Skin testing loses its value in dermographic individuals, and a RAST is necessary in such persons to measure the subject's propensity to make IgE against specific antigens (2).

Test results are read 15 min after the skin pricks have been made. Some persons will not have a maximal test response at 15 min, and the test sites should be reviewed at 30 min in these individuals. Tests are read by measuring the largest and smallest diameters of the wheal and erythema individually using a millimeter ruler. The average of the two measured diameters of the wheal and erythema, respectively, are used in scoring the test results. Commonly, the grading system of Norman and colleagues is used in skin test interpretation (Table 1).

Many allergists will proceed to intradermal testing in patients who have negative prick skin tests, to

determine if the subject has an allergen sensitivity below that which is detectable by the prick test and yet may still be clinically relevant. The skin is prepared in the same fashion as for prick testing with proper cleansing. Sterile, disposable 26-gauge tuberculin syringes are used for intradermal testing and are filled with 0.1 ml of the test solution, ensuring that no air bubbles are in the syringe. The skin of the testing site should be pulled taut by the technician, and the needle of the syringe is introduced into the skin with the bevel down and the syringe at a 45° angle to the skin surface. The needle tip is inserted into the skin, and the tip is lifted gently as to pick up the skin with the tip. The syringe is then lowered to the level of the skin to eliminate the angle that had been present on skin insertion, and the needle is advanced into the skin so that the bevel entirely penetrates the skin, but remains within the layers of the skin to avoid subcutaneous administration of the skin test solution. The fluid in the syringe is then injected into the skin such that a small wheal of about 3 mm in diameter is formed. If an error occurs in the administration of the test solution, a new site should be chosen so that one can have confidence in the test results. Again, the skin reactions are read 15 min after the tests have been placed. Allergists who perform intradermal testing often will perform intradermal tests with serial dilutions of extract material to determine the relative sensitivity of a patient for a particular antigen, as such information may be helpful in determining concentrations of extracts to be used in allergen immunotherapy. Serial dilutions are made from commercially produced concentrated extracts in a 10-fold dilution series. The diluting fluid is usually phosphate-buffered physiologic saline at pH 7.4, with 0.4% phenol to inhibit bacterial growth. New transfer pipettes or syringes are used in creating each dilution, as extract material could be carried over from previous more concentrated dilutions and significantly alter the concentration of a dilution. A 50% glycerol concentration in the undiluted extract increases its stability from degradation, and 0.03% human serum albumin can be added to prevent adsorption of the extract to the glass vial in which the extract dilutions are stored (2).

Skin reactivity may vary between two individuals who have the same quantity of allergen-specific IgE in their blood and may also vary between different test sites and different testing times in the same person. These factors are both dependent on the allergen being used, as well as upon specific character-

TABLE 1

Grading System for Skin Test Interpretation (31)

Grade	Erythema	Wheal
0	<5 mm	<5 mm
±	5–10 mm	5–10 mm
1+	11–20 mm	5–10 mm
2+	21–30 mm	5–10 mm
3+	31–40 mm	5–10 mm or with pseudopods
4+	>40 mm	>15 mm or with many pseudopods

istics of the patient or patients being tested. The number of IgE-binding epitopes present on a particular antigen, the affinity of a individual's IgE molecules for those epitopes, the number of mast cells at a test site, the ability of those mast cells to release mediators, the ability of the skin to respond to those mediators, and the ability of the skin to degrade an allergen are all important factors in determining the skin test reaction (3). Witteman and colleagues concluded that these allergen- and patient-dependent factors are important in the significant relationship between the threshold at which a serial dilution of a purified allergen extract causes a positive intradermal test and the specific IgE concentration of that purified allergen measured in blood by RAST (3). In their study, the threshold of a positive skin test was defined as a wheal of 5 mm and an erythema of 10 mm, and a very strong correlation ($r = 0.98$; $P < 0.001$) between the wheal and the flare thresholds was noted. The authors found that at higher specific IgE concentrations, the degree of skin test reactivity within an individual plateaued, thus making the size of a single skin test reaction a poor predictor of the amount of antigen-specific IgE in blood. Although the relationship between the dilution of allergen extract that caused a threshold positive skin test and the antigen-specific IgE level was significant in the subject population, the dilution of antigen that was necessary for a positive skin test could vary by as much as a factor of 100 in subjects with similar blood antigen-specific IgE levels. One factor that may contribute to this high intersubject variability in the threshold skin test dilution is a subject's total IgE level. Witteman *et al.* found that there was a significant inhibitory effect of total serum IgE on specific allergen skin test sensitivity, perhaps a result of competition of the irrelevant IgE with specific IgE for IgE-binding receptors on mast cells (3). Of clinical significance to practicing allergists, the authors found there were no positive skin tests when less than 0.01 pg of antigen was used, despite high levels of allergen-specific IgE in the subjects being tested, thus creating a guidepost for the lowest concentration of extract needed for skin testing (3).

The use of intradermal skin testing is somewhat controversial among practicing allergists. Intradermal tests are usually performed only when prick tests are negative, with the rationale that they are more sensitive. However, the clinical relevance of this additional information of lower level sensitivity is questionable. Nelson *et al.* recently examined the value of intradermal skin testing in the diagnosis of clinically

important sensitivity to timothy and related grass pollens in subjects who had negative prick skin tests (4). Subjects with symptoms of allergic rhinitis were identified who had positive prick tests to timothy grass, negative prick tests and positive intradermal tests to timothy grass, or both negative prick and intradermal tests to timothy grass. Timothy prick testing was performed with 100,000 allergen units (AU)/ml, and intradermal testing was executed with 1000 AU/ml. There were both significantly greater numbers of positive nasal challenges to timothy and increased symptoms during the timothy season in those subjects who had positive prick tests compared to the two groups that had negative prick tests. There was no difference between the prick negative, intradermal positive and the prick negative, intradermal negative groups in the results of either the timothy nasal challenges or the symptom scores during the timothy season. The authors concluded that positive intradermal skin tests alone to timothy grass do not indicate clinically significant sensitivity in subjects who had negative prick tests (4).

The ability of skin tests to accurately determine exacerbating factors for allergic rhinitis symptoms is clinically important in diagnosing and treating this disease. Although many investigators have previously analyzed this issue, Droste and colleagues recently reported their examination of the association of skin test reactivity, antigen-specific IgE, and total IgE with rhinitis symptoms in a community-based population study (5). The allergens used in this report were dust mite (*Dermatophagoides pteronyssinus*), cat, molds (*Cladosporium herbarum* and *Alternaria alternata*), timothy grass, olive, birch, *Parietaria spp.*, and common ragweed. In this study, the authors found that both skin test positivity and serum-specific IgE were about equally closely related to rhinitis symptoms in response to allergen exposure and that there was an increased relationship of positive test results to allergy symptoms when these two tests were combined. The authors attribute the similarity of results between skin tests and serum-specific IgE to the use of a CAP system, whereas previous investigators had found a less striking relationship of serum-specific IgE as measured by RAST to symptom scores, compared to relationship of skin testing with symptoms. The association of both skin test reactivity and serum-specific IgE with nasal symptoms was strongest for cat allergen, and a significant association between the two tests and symptoms also existed with all of the other allergens, excluding *Cladosporium*. Only high levels

of total IgE (>87.6 kU/L) were independently related to nasal symptoms. High total IgE levels were also predictive of future skin test positivity when subjects are retested 5 years later, and lower IgE levels in subjects initially skin test positive were predictive of loss of skin test reactivity after 5 years (5).

In addition to determining allergic sensitization to natural aeroallergens, skin testing is useful in diagnosing IgE-mediated hypersensitivity to latex, insect venoms, medications, and acid anhydrides. Over the past few years, latex has been recognized as a significant cause of type I hypersensitivity reactions among health care personnel and patients with spina bifida. However, it has been difficult to prepare test extracts to accurately diagnose latex allergy because of the various antigenic profiles in different latex-containing products, as well as the interindividual specificities of anti-latex IgE antibodies (6). Hamilton and colleagues examined non-ammoniated latex, ammoniated latex, and latex rubber gloves as sources of skin testing extracts (7). In this study, ammoniated and nonammoniated latex was prepared from crude *Hevea brasiliensis* tree sap. The glove extract was prepared by pipetting sterile phosphate-buffered saline solution (5 ml per gram of glove) into natural rubber latex gloves. The wrist opening of the glove was knotted closed so that the fluid would remain in the glove, and each was agitated on a shaker for 16 h at 21°C. After this incubation process, the fluid was removed from the glove, centrifuged (1000g for 10 min) to pellet the powder that had lined the inside of the glove, and decanted from the pellet. The liquid material was then concentrated to 1.7 mg/ml and sterile-filtered before being used as the glove extract skin test material. Although there were equivocal diagnostic sensitivities and specificities with the three extracts after they had been normalized to milligrams per milliliter of total protein, the investigators concluded that the nonammoniated reagent was the reagent of first choice based on consistency between lots, long-term stability, and the ease of immunochemical quality control. The specificity of the nonammoniated extract was 96% for puncture and 93% for intradermal testing, while the specificities were 100% for the puncture and 96% for the intradermal tests. The optimal diagnostic accuracy was achieved at 100 μ g/ml for puncture tests and 1 μ g/ml for intradermal tests (7). Recently, Hadjiliadis and colleagues reported their experience with a commercial skin prick test solution available in Canada (low ammoniated raw latex solution obtained from Bencard Labora-

tories, Mississauga, Ontario, Canada) in which they found that there was an association between the size of the skin test response and the severity of latex-induced symptoms (8). Commercial extracts are still not available for skin testing in the United States, but a multicenter phase 3 study with a candidate nonammoniated latex preparation is under way based on the safety and efficacy data that have been published (7).

Skin testing is also widely used in the United States to diagnose allergic reactions to insect stings. The availability of purified insect venoms has greatly advanced accurate diagnosis and treatment of this form of hypersensitivity; however, several controversies remain in this field. For a patient who has suffered a systemic reaction from a sting from an unseen insect, Reisman recommends intradermal skin tests with a 0.02-ml volume to all five commercially available venom extracts at an initial concentration of 0.001 μ g/ml (9). These five extracts are bald faced hornet, yellow hornet, yellow jacket, *Polistes*, and honeybee venoms. If the results of the 0.001 μ g/ml dose are negative, then Reisman recommends performing intradermal skin tests with increasing doses to 0.1 μ g/ml. He cautions that intradermal use of a concentration of 1.0 μ g/ml or greater must be carefully assessed for clinical significance, as this dose may blur the distinction between immunologically specific reactions and irritant reactions. Caution must also be taken in interpreting positive skin tests to *Polistes* at any concentration, as these may result from cross-reactivity among other venom proteins. Hamilton and colleagues developed a venom RAST inhibition assay to help distinguish cross-reactive venom-specific IgE (10). Their study showed that one-third of patients who had both positive yellow jacket and *Polistes* skin tests did not need *Polistes* in their immunotherapy regimen, because the patient's IgE that recognizes *Polistes* was $>95\%$ inhibitable with yellow jacket venom. In other words, the patient's positive skin test to *Polistes* was only a result of cross-reactivity of the patient's anti-yellow-jacket IgE antibody for *Polistes*, not a true sensitivity to *Polistes*. The authors found that this serologic analysis in patients who had positive skin tests to both yellow jacket and *Polistes* was cost-effective in eliminating an expensive extract from therapy that was not truly needed. The use of skin testing to diagnose clinically relevant hypersensitivity to venom, that is to predict who is at risk for future systemic reactions and treat those persons with venom immunotherapy, is much more contro-

versial in Europe. van Halteren and colleagues propose that in-hospital sting challenges with live insects under intensive care conditions appear to have the highest negative predictive value. In a recent report, they followed 348 patients who had experienced systemic reactions to insect stings and who subsequently had negative reactions to in-hospital sting challenge and therefore were not given venom immunotherapy (11). Of 129 of these patients who had been restung, only 6 experienced serious manifestations and only 2 suffered hypotension, but none died. These authors believe that immunotherapy is overprescribed when it is based on skin test results and that this leads to unnecessary commitments of both cost and time for those patients who would have a negative sting challenge. They note that there have been no deaths reported in the literature after insect sting challenge. However, investigators at Johns Hopkins University treated two near fatal reactions while performing sting challenges on patients who had received placebo immunotherapy, reactions that left a lasting impression that such sting challenges are extremely high-risk procedures (12).

Skin testing is also valuable in predicting immediate-type hypersensitivity reactions to a few medications. Currently, the only group of antibiotics for which skin testing has been validated for its predictive value is the β -lactams. Given the widespread use of β -lactams, the relatively high rate of sensitization to these medications, and the possible fatal outcome of a severe reaction, accurately diagnosing the propensity for future allergic reactions to this class of antibiotic is very important. Current recommendations are to use both major and minor determinant penicillin derivatives in skin testing, first with a prick test, and if the prick test is negative, then with intradermal tests (13). Skin testing to both the major determinant (oat-benzylpenicilloyloctalysine) and the minor determinant mixtures (potassium benzylpenicillin, benzylpenicilloylate, and benzylpenicilloyl-*N*-propylamine) is needed to avoid false-negative reactions when only one or the other skin testing reagent is used. The predictive value of skin testing other antibiotic classes has not been fully studied, and the reliability is not as great as with the β -lactams (14). However, a protocol that might have some utility in diagnosing sensitivity to other antibiotics involves making three serial 10-fold dilutions of a 1 mg/ml solution of the antibiotic in question in sterile buffered saline. Puncture testing is performed with the 1:1000 dilution, along with the appropriate histamine and saline controls.

Should the puncture test be negative, intradermal testing is then initiated starting with the lowest concentration and increasing until a positive skin test is observed. If a positive test develops, testing with the same dose that caused a positive response should be performed on control subjects to ensure that the reaction was not solely an irritant response. If the skin tests of the antibiotic dilutions performed on the original subject are negative, then a skin test with undiluted antibiotic (up to 20 mg/ml) can be performed. If a test is positive in the original subject and the control subjects have negative tests, then the patient may indeed make clinically significant amounts of IgE against that antibiotic. However, the predictive value of negative skin tests in such a situation is unknown (14). Skin testing has also been performed with local anesthetics with a great degree of reliability. The value of skin tests in this situation is to determine the nature of an adverse reaction that occurred while receiving that medication class in the past. Since local anesthetics are often given prior to an uncomfortable procedure, it is often difficult to determine whether an adverse reaction associated with local anesthetics is related to the sympathetic response from the fear of the impending procedure, is related to the discomfort of the procedure itself, or is a truly immunologically mediated reaction from the medication. Type I hypersensitivity reactions to local anesthetics are extremely rare, and negative skin tests allow an important class of medications to be used safely thereafter (15). Many investigators feel that it is necessary to use several placebo skin tests in the course of skin testing to anesthetics to accurately assess whether a reaction may be related to the fear of the medication rather than the medication itself.

Acid anhydrides, materials used in the production of resins, varnishes, and adhesives, have been attributed to cause type I hypersensitivity reactions such as conjunctivitis, rhinitis, asthma, and urticaria. Baur and Czuppon have recently reported anhydride extracts that are suitable for skin testing, as well as for nasal and bronchial provocation challenges and enzyme-allergosorbent tests (EAST) (16). The authors prepared conjugates of human serum albumin with phthalic, maleic, trimellitic, and pyromellitic anhydrides and tested them in workers with respiratory symptoms on exposure to the anhydrides. They found that although these anhydride conjugates were useful in the *in vivo* provocation studies, the EAST had superior sensitivity to the skin tests (16).

NASAL PROVOCATION

Although skin tests represent the major diagnostic procedure that allergists employ after taking the patient history and performing a physical examination, nasal challenges are most often restricted to research. There are several methods of nasal challenge currently in use by different research centers, and these methods will be outlined.

Naclerio and colleagues have used both a nasal lavage model and a filter paper disc model to measure the biologic and physiologic responses to antigen challenge in allergic rhinitis patients (17). The nasal lavage model utilizes insufflation of the antigenic material into the nose, followed by serial nasal lavages to collect mediators released by the response to allergen challenge (18). Prior to the challenge procedure, the subject is prepared with four nasal prewashes (lavage technique described below) to reduce existing cell-free mediator concentrations to a baseline level. This is followed by administration of topical oxymetazoline, a substance shown not to affect mediator release, but that prevents the mucosal edema that restricts the amounts of lavage fluid that can be instilled in the subject's nose (19). In this model, specific doses of dry pollens are combined with lactose to obtain a total weight of 28.4 mg, and the mixture is then placed in a gelatin capsule (18). The capsule is placed in a spinhaler adapted for delivery of material to the nares, and the spinhaler is connected to a French-Rosenthal dosimeter. The dosimeter is activated for 2 s four times with a driving pressure of 10 pounds per square inch so that about 25 mg of pollen and lactose are propelled from the spinhaler into the nose. When the use of liquid allergen extracts is desired, these extracts are placed in a DeVilbiss 646 nebulizer that is adapted to fit the nostril. The nebulizer is also attached to a French-Rosenthal dosimeter so that 0.08 ml of extract is delivered to the nose. Doses of allergen have varied but range in increasing doses from 10 to 1000 protein nitrogen units.

Following delivery of a dose of pollen or extract, the subject undergoes nasal lavage. In this procedure, the subject sits upright in a chair and extends his or her neck to an angle of 30° from horizontal. After the subject is instructed not to breathe or swallow during the lavage, 5 ml of normal saline is instilled into each nostril. After 10 s, the subject flexes his or her head and expels the mixture of mucus and saline into a container that is stored on ice until the experiment is completed. Afterward, the material

collected is centrifuged. The supernatant is assayed for mediators, and the gelatinous phase can be examined for differential cell counts (18).

The disc model was developed because of several recognized problems with the lavage method (17). First, subjects are premedicated with oxymetazoline to prevent the mucosal edema that results from antigen challenge, thus preventing physiologic measurements such as airway resistance. Second, the model was unable to differentiate the products of one side of the nose compared to the other when there was a challenge of only one side as the products of the allergic reaction would mix in the back of the nasopharynx with the lavage procedure. The disc model consists of placing an allergen-impregnated white filter paper disc on the nasal septum. These discs can both administer allergen to the nasal mucosa and serve as a vehicle to collect secretions at predetermined time intervals afterward. To measure the amount of secretions produced by the challenge, the disc and an Eppendorf tube are weighed together prior to the challenge, the disc is removed from the nasal cavity after challenge and placed in the Eppendorf tube, and the disc and Eppendorf tube are reweighed. Mediators can be eluted from the disc and quantified (20). This method also allows for measurement of secretion and mediator release from the contralateral side of the nose to determine reflex activity that occurs as a result of challenge. Mediators that have been measured using these models include histamine, prostaglandin D₂, kinin, albumin, and TAME-esterase (17, 20, 21). Nasal responses to materials other than allergens can also be made. Discs impregnated with histamine and methacholine have been used to measure the ability that various pharmacologic agents have in blocking physiologic responses to these two substances that cause rhinitis symptoms (20).

Other investigators have used direct delivery of allergen via nasal spray bottles containing the desired dose of the challenge material (22). This method simplifies the procedure but suffers in that the exact quantity of material delivered may vary substantially from spray to spray. Depending on the nature of the study, most investigators recommend discontinuing topical corticosteroids and cromolyn sodium for 2 weeks prior to challenge and systemic corticosteroids for 4 weeks before the study protocol.

The results from nasal challenges can be established from either the concentrations of the mediators released or the symptoms experienced as a consequence of the challenge. Components of the

symptom severity scores include the number of sneezes, the presence or absence of rhinorrhea, itchy eyes, nasal congestion, and throat irritation (21).

BRONCHIAL PROVOCATION

A subject with allergic asthma will have lower respiratory tract symptoms when exposed to an aerosolized antigen to which that person has a positive skin test if the antigen is given in sufficient quantity. However, there are instances in which a person with allergic asthma will not have asthma symptoms to an antigen to which that subject is skin test positive. Bronchial provocation allows an investigator to determine whether an antigen to which a subject has significant specific IgE, either by skin test reactivity or RAST, specifically causes lower airway symptoms (2). In its simplest form, whole lung antigen challenge consists of a subject breathing increasing doses of antigen until the capacity to exhale forcefully is diminished by one-fifth of that person's normal capability. Whole lung antigen challenge essentially creates a mild to moderate asthma exacerbation and as such should be performed in a setting where emergency equipment is available in the event of severe respiratory compromise. Whole lung antigen challenge should not be performed when the subject is in the midst of respiratory compromise, as determined by significant wheezing on physical exam or by pulmonary function testing. Most physicians will not perform whole lung antigen challenge if the forced expiratory volume at 1 s (FEV_1) is under 60% of predicted for that subject's age, height, and gender. Generally, this technique is reserved for research settings; however, whole lung antigen challenge can be used in the clinic setting to confirm the supposition that a specific allergen may be significantly contributing to a patient's respiratory symptoms (23).

The dosage of antigen at which whole lung antigen challenge is initiated can be determined by skin testing serial 10-fold dilutions of an allergen extract to which one makes IgE antibody. The threshold dose at which a person has a 10-mm wheal is the initial dose at which whole lung antigen challenge can be safely started (2). The most widely used devices for aerosolization of allergen extracts are the Wright and DeVilbiss nebulizers. When either the Wright or the DeVilbiss nebulizer is regulated by a dosimeter, the reproducibility of the results in the same subject is similar (24).

Before the procedure is initiated, baseline pulmonary function tests are performed and the FEV_1 is recorded. The subject then takes five breaths of the solution used as the diluent for the allergen extract, usually sterile 10% phosphate-buffered saline, to assess that individual's degree of nonspecific reactivity. Three minutes after this control challenge, spirometry is performed. Should the FEV_1 decrease by greater than 10% from the prediluent challenge baseline, the study may be terminated as the specificity of the inhaled allergen challenge will not be able to be reliably assessed. If the nonspecific reactivity from the diluent inhalation is not great, then the subject takes five breaths of the initial allergen dilution as determined by the threshold skin test titration. Ten minutes later, spirometry is again performed and the FEV_1 is noted. This same cycle of inhaled allergen challenge, waiting 10 min, measuring spirometry, and assessing the fall in FEV_1 is repeated with half-log increasing concentrations of allergen until there is a 20% decrease in the FEV_1 from the postdiluent challenge FEV_1 or until the maximum dose of allergen has been given (23).

The top dose of allergen to be given must be carefully determined and will possibly vary for each allergen. Very large concentrations of inhaled allergen will precipitate asthma symptoms in allergic rhinitis subjects who may never previously experienced such symptoms. The purpose of the challenge is to ascertain whether a cause-and-effect relationship exists between a particular allergen and asthma symptoms. A false-positive reaction can result from too high a dose. In our whole lung antigen challenges, we have used 20,000 protein nitrogen units as the highest concentration of inhaled allergen (2).

After there has been a decrease in the FEV_1 by at least 20%, the dose of allergen that caused the threshold of the immediate response can be calculated. If a Wright nebulizer were utilized, the end point would be expressed as the provocative concentration that elicited a drop in FEV_1 of 20% from the FEV_1 following diluent control (PC_{20}). If a DeVilbiss nebulizer were used, the end point would be expressed as the provocative dose that produced a 20% fall in FEV_1 from the postdiluent baseline (PD_{20}). In general, the PC_{20} is reported as a noncumulative measurement in milligrams per millimeter. The PD_{20} is most often expressed as a cumulative dose, in either micromoles or "breath units." The number of breath units is calculated by the following formula: breath units = concentration ($\mu\text{g/ml}$) times the number of breaths. The results of challenges ei-

ther with the Wright or with the DeVilbiss nebulizer are ascertained by a dose-response curve using a semilogarithmic scale. The dose (either cumulative or noncumulative) of the antigen used is plotted on the logarithmic abscissa, and the FEV₁ responses as a percentage of the diluent control inhalation are plotted on the linear ordinate (see Fig. 1). The best line fit for the values obtained is determined, and the PC₂₀ or PD₂₀ is calculated by interpolating from the final two concentrations or doses the point at which the FEV₁ is 20% decreased (24).

When a 20% or more decrease in FEV₁ has occurred, the airway response can be reversed with a bronchodilator or assessment of a possible late-phase reaction can be made by performing hourly spirometry measurements for 8 h. The late-phase reaction in the lung is defined as an FEV₁ value measured 4 to 8 h after the completion of the whole lung challenge that is less than 15% of the FEV₁ measured after diluent control. Roughly 30 to 40% of allergic asthmatics who receive enough inhaled allergen to precipitate an immediate reaction will experience a late-phase reaction. Although the physiologic mechanisms that cause allergen-induced

late-phase reactions in the lung are not fully defined, three factors that are believed to contribute to these reactions are smooth muscle constriction, airway edema, and increased mucous production (2).

Asthma medications can inhibit whole lung antigen challenges and should be avoided for the period just prior to the challenge so that the test will have the greatest degree of reliability (2). Guidelines for the length of time that these medications should be avoided before the challenge are listed in Table 2.

While the whole lung allergen challenge measures bronchial reactivity to a specific allergen, methacholine and histamine challenges measure nonspecific bronchial reactivity. These tests are not specific for the diagnosis of asthma; however, the methacholine and histamine challenges are often used in evaluating patients who relate symptoms of asthma, but who have never been observed during an exacerbation of their condition. The contraindications of not performing methacholine or histamine challenges when the subject is in the midst of respiratory compromise, as determined by significant wheezing on physical exam or by an FEV₁ <60% of predicted, are the same as with whole lung antigen challenge (2).

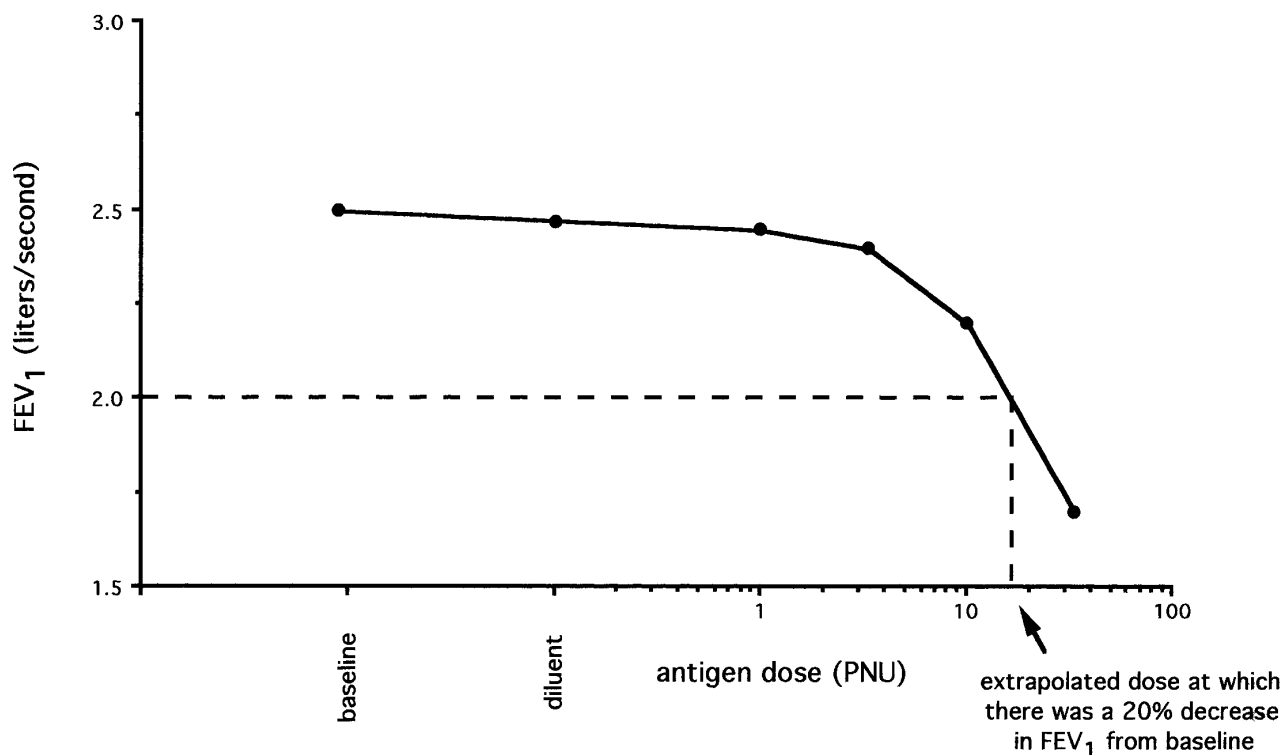


FIG. 1. Graph of the method of determining the dose of antigen at which there is a positive whole lung antigen challenge, when a positive challenge is defined as a fall in the FEV₁ by 20% from the diluent challenge.

Methacholine directly interacts with muscarinic receptors to effect smooth muscle contraction. The peak of methacholine action results within 1 to 4 min after inhalation, and the duration of activity is between 1 and 2 h. Tachyphylaxis (a decrease in response when doses are repeated) does not occur during methacholine challenges, although there is diurnal variability of up to one to two doubling doses in causing a 20% fall in FEV₁. Although methacholine is very safe when administered properly, occasional subjects will experience flushing at higher concentrations. Methacholine is relatively inexpensive and stable in solution for up to 3 months. Methacholine is dosed at half-log increasing concentrations with measurements of FEV₁ made 3 min after the end of inhalation of each dose. A common starting dose is 0.025 mg/ml, and most laboratories do not proceed past a dose of 25 mg/ml (2). Perpina and colleagues reported that a PC₂₀ of 15 mg/ml for distinguishing asthmatic from nonasthmatic patients has a sensitivity of 84% with a specificity of 86% (25).

Histamine's peak activity is also between 1 and 4 min; however, the duration of activity is only 10 to 30 min. Tachyphylaxis can occur with histamine and has been reported to last up to 6 h. Histamine challenge is also subject to the same degree of diurnal variation as methacholine, and it is safe when administered properly, but it is associated with the side effects of flushing, headache, and tachycardia more frequently than methacholine. Histamine is relatively inexpensive and stable in solution for up to 3 months (2). Cockcroft and colleagues reported that a PC₂₀ to histamine of 8 mg/ml was 100% sensitive, with a specificity of 93% for a group of college students "with current symptomatic asthma" (26).

TABLE 2

Period of Abstinence from Various Medications Prior to Bronchial Inhalational Challenges to Avoid Confounding Impact of Medication on Challenge

Medication	Time
Inhaled short-acting β -agonist and anticholinergic agents	8 h
Oral β -agonists	24 h
Theophylline preparations, salmeterol	48 h
Classical antihistamines	72 h
Terfenadine	5 days
Inhaled cromolyn and nedocromil, nasal cromolyn	1 week
Inhaled and nasal corticosteroids	2 weeks
Oral corticosteroids	4 weeks
Astemizole	8 weeks

The reproducibility of allergen, methacholine, and histamine challenges depends on the technique employed and the health of the subject (2). Subjects are not routinely studied during the course of asthmatic exacerbations for two reasons. First, environmental allergen exposure to substances such as animal dander or pollens increases nonspecific bronchial reactivity, and therefore the results can be confounded by these exposures. Second, environmental allergen priming could potentiate the bronchial responsiveness to lower allergen doses, thus potentially resulting in excessive responses that would not normally occur out of season, which decreases safety. Subjects should not have an upper respiratory tract infection, such as bronchitis, sinus infection, or viral infection, for at least 2 weeks prior to challenge, as these also increase nonspecific bronchial reactivity.

Allergic disease has long been associated with bronchial reactivity. Although there is a poor correlation between skin test reactivity and concentration of histamine that results in a positive bronchial challenge, the best predictor for bronchial responsiveness to whole lung antigen challenge is a combination of both skin test and histamine challenge results through multiple linear regression (27). This relationship suggests that bronchial responsiveness to aeroallergen challenge is dependent on both allergen sensitivity and degree of nonspecific bronchial responsiveness.

There have been several recent studies exploring the relationships between skin test positivity and specific IgE levels with bronchial responsiveness to either methacholine or histamine. Burrows and co-workers found that although overall bronchial hyperresponsiveness to methacholine decreased with age in a cohort followed from ages 9 to 15, the tendency to have hyperresponsiveness to methacholine and retain increased responsiveness over time was closely related to high serum IgE levels and positive skin tests (28). They also concluded that a single measurement of bronchial responsiveness at any point in time did not accurately reflect the tendency for bronchial hyperreactivity. Burrows and colleagues also recently reported that positive skin tests to dust mites (*D. pteronyssinus*), cat, dog, and *Aspergillus fumigatus* related univariately to bronchial responsiveness in 13-year-old children, while specific IgE levels of these allergens and skin test and IgE levels to mixed rye grass, horse, kapok, *Alternaria*, *Penicillium*, and *Cladosporium* did not (29). The authors caution that they do not imply that bronchial reactivity is a result of allergy to certain aeroallergens as exposure history was not available.

They point out that skin testing appears to be a better predictor of nonspecific reactivity than serologic measurements of specific IgE. Renstrom *et al.* recently reported that bronchial responsiveness to methacholine did not predict allergic sensitization in animal care workers (30). In this study, the workers had no history of asthma or positive skin tests to animals prior to starting their new jobs as laboratory technicians handling animals. However, a preemployment IgE level of greater than 100 kU/L predicted the onset of airway symptoms to animals after a median follow-up of 18 months of work in the animal area. This study has significant implications for both prospective workers with high IgE levels who may work in animal facilities and for the institutions that hire them with regard to future disability.

In conclusion, *in vivo* testing for allergic sensitization provides important information for the diagnosis of disease, and it also offers the opportunity to test the effectiveness of new pharmaceutical agents in a controlled setting. In addition, the knowledge from these tests can allow health care workers to predict who is at risk for future difficulties related to allergen exposures.

REFERENCES

1. Sutton, B. J., and Gould, H. J. (1993) *Nature* **366**, 421–428.
2. Norman, P. S., and Peebles, R. S., Jr. (in press) in *Manual of Clinical Laboratory Immunology* (Rose, N. R., Conway de Macario, E., Folds, J. E., Lane, H. C., and Nakamura, R. M., Eds.), ASM Press, Washington, DC.
3. Witteman, A. M., Stapel, S. O., Perdok, G. J., Sjamsoedin, D. H., Jansen, H. M., Aalberse, R. C., and van der Zee, J. S. (1996) *J. Allergy Clin. Immunol.* **97**, 16–25.
4. Nelson, H. S., Oppenheimer, J., Buchmeier, A., Kordash, T. R., and Freshwater, L. L. (1996) *J. Allergy Clin. Immunol.* **97**, 1193–1201.
5. Droste, J. H., Kerhof, M., de Monchy J. G., Schouten, J. P., and Rijcken, B. (1996) *J. Allergy Clin. Immunol.* **97**, 922–932.
6. Tomazic, V. J., Withrow, T. J., and Hamilton, R. G. (1995) *J. Allergy Clin. Immunol.* **96**, 635–642.
7. Hamilton, R. G., and Adkinson, N. F., Jr. (1996) *J. Allergy Clin. Immunol.* **98**, 872–883.
8. Hadjiliadis, D., Banks, D. E., and Tarlo, S. M. (1996) *Am. J. Respir. Crit. Care Med.* **97**, 1202–1206.
9. Charous, B. L., Hamilton, R. G., and Yunginger, J. W. (1994) *J. Allergy Clin. Immunol.* **94**, 12–18.
10. Hamilton, R. G., Wisenauer, J. A., Golden, D. B., Valentine, M. D., and Adkinson, N. F., Jr. (1993) *J. Allergy Clin. Immunol.* **92**, 651–659.
11. van Halteren, H. K., van der Linden, P. W., Burgers, S. A., and Bartelink, A. K. (1996) *J. Allergy Clin. Immunol.* **97**, 1058–1063.
12. Smith, P. L., Kagey-Sobotka, A., Bleeker, E. R., Traaystman, R., Kaplan, A. P., Gralnick, H., Valentine, M. D., Permutt, S., and Lichtenstein, L. M. (1980) *J. Clin. Invest.* **66**, 1072–1080.
13. Sogn, D. D., Evans, R., III, Shepherd, G. M., Casale, T. B., Condemi, J., Greenberger, P. A., Kohler, P. F., Saxon, A., Summers, R. J., VanArsdel, P. P., Jr., *et al.* (1992) *Arch. Intern. Med.* **152**, 1025–1032.
14. Peebles, R. S., Jr., and Adkinson, N. F., Jr. (1996) in *Current Therapy of Infectious Disease* (Schlossberg, D., Ed.), pp. 617–621, Mosby-Year Book, St. Louis, MO.
15. Chandler, M. J., Grammar, L. C., and Patterson, R. (1987) *J. Allergy Clin. Immunol.* **79**, 883–886.
16. Baur, X., and Czuppon, A. (1995) *J. Allergy Clin. Immunol.* **96**, 489–494.
17. Naclerio, R. M. (1992) *J. Allergy Clin. Immunol.* **90**, 715–719.
18. Naclerio, R. M., Meier, H. L., Kagey-Sobotka, A., Adkinson, N. F., Jr., Meyers, D. A., Norman, P. S., and Lichtenstein, L. M. (1983) *Am. Rev. Respir. Dis.* **128**, 597–602.
19. Majchel, A. M., Baroody, F., Kagey-Sobotka, A., Lichtenstein, L. M., and Naclerio, R. M. (1993) *J. Allergy Clin. Immunol.* **92**, 767–770.
20. Wagenmann, M., Baroody, F. M., Kagey-Sobotka, A., Lichtenstein, L. M., and Naclerio, R. M. (1994) *J. Allergy Clin. Immunol.* **93**, 594–605.
21. Naclerio, R. M., Proud, D., Togias, A. G., Adkinson, N. F., Jr., Meyers, D. A., Kagey-Sobotka, A., Plaut, M., Norman, P. S., and Lichtenstein, L. M. (1985) *N. Engl. J. Med.* **313**, 65–70.
22. Stanaland, B. E., Fernandez-Caldas, E., Jacinto, C. M., Trudeau, W. L., and Lockey, R. F. (1996) *J. Allergy Clin. Immunol.* **97**, 1045–1049.
23. Spector, S. L. (1989) in *Provocative Challenge Procedures* (Spector, S. L., Ed.), pp. 293–339, Futura, Mount Kisco, NY.
24. Ryan, G., Dolovich, M. B., Roberts, R. S., Frith, P. A., Juniper, E. F., Hargreave, F. E., and Newhouse, M. T. (1981) *Am. Rev. Respir. Dis.* **123**, 195–199.
25. Perpina, M., Pellicer, C., de Diego, A., Compte, L., and Macian, V. (1993) *Chest* **104**, 149–154.
26. Cockcroft, D. W., Murdock, K. Y., Berschied, B. A., and Gore, B. P. (1992) *J. Allergy Clin. Immunol.* **89**, 23–30.
27. Cockcroft, D. W., Ruffin, R. E., Frith, P. A., Cartier, A., Juniper, E. F., Dolovich, J., and Hargreave, F. E. (1979) *Am. Rev. Respir. Dis.* **120**, 1053–1058.
28. Burrows, B., Sears, M. R., Flannery, E. M., Herbison, G. P., Holdaway, M. D., and Silva, P. A. (1995) *Am. J. Respir. Crit. Care Med.* **152**, 1302–1308.
29. Burrows, B., Sears, M. R., Flannery, E. M., Herbison, G. P., and Holdaway, M. D. (1995) *J. Allergy Clin. Immunol.* **95**, 548–556.
30. Renstrom, A., Malmberg, P., Larsson, K., Larsson, P. H., and Sundblad, B. M. (1995) *Eur. Respir. J.* **8**, 1514–1519.
31. Norman, P. S. (1983) in *Allergy, Principles and Practice* (Middleton, E., Jr., Ellis, E. F., and Reed, C. E., Eds.), pp. 295–302, Mosby-Yearbook, St. Louis, MO.