

SKIN TESTS

A Historic Trail

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SKIN AS A TISSUE REFLECTOR OF IMMUNE PHENOMENA

With early moves to shape allergy as a separate segment of medicine,³⁶ the clinical entities within its defined scope were placed within a group variously referred to as *clinical anaphylaxis*, *human hypersensitivity*, and *protein sensitization*. Sharing the pathogenetic commonality of untoward responses to substances of otherwise innocuous character, their categorization was derived from the concept of putative relationship to experimental animal phenomena reported from German and French laboratories. In particular, counterpart identifications centered on discoveries of tissue hyper-reactivity to reinfection with tubercle bacilli,⁹³ systemic anaphylaxis,¹²⁸ and local anaphylactic manifestations.⁸

Nomenclature that came into common usage introduced a mix of confusing descriptors, including Behring's "hypersensitivity" (*überempfindlichkeit*) to denote enhanced, injurious responses (increasing susceptibility) to small repeated immunizing doses of diphtheria toxin¹²; Von Pirquet's "allergie" (Greek *allos*—other, *ergos*—effect) that covered harmful and beneficial altered reactivity¹²⁵; and Doerr's use of "hypersensitivity" for classification of diverse forms of reactions that differed from normal physiologic responses.⁵³ To differentiate the aggregate of serum disease, contact dermatitis, anaphylaxis, and hypersensitivity from the

Amy Betz, BA, BS and Vivian Tawadros, BA, BS, as pre-professional school participants in the NIH Summer Student Program, assisted in the research of archival material in the development and preparation of this article.

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clinical entities of asthma, hay fever, and eczema, American pioneering immunologist Arthur Coca designed a new categorical classification. In naming the latter group "atopy," Coca related their shared character to the Greek word *atopia* (strangeness) to denote the "strange" quality of disorders identified with adverse reactions to otherwise innocuous foods, pollens, and perennial inhalants.³²

In retrospect, observations on strange human deviations—subsequently recognized as hay fever and asthma—had been recorded 4 centuries earlier. In 1552, Giralamo Cardana, an Italian professor of medicine at Pavia, in the invited capacity of visiting consultant, resolved the Scottish Archbishop John Hamilton's problem of nocturnal asthma by eliminating his feather-stuffed pillows and mattress.²⁶ In 1567, the Jewish Portuguese physician and medical botanist João Rodriguez de Castello Branco, also known as Amatus Lusitanus—after fleeing from Portugal's Inquisition to Italy and a professorship in medicine at Ferrara—provided the account of a monk whose nasal symptoms kept him indoors during the rose-blooming season.⁷ In a similar circumstance in 1565, Leonardo Botallo, an Italian anatomist in the French royal medical service, described a patient who suffered from sneezing fits and running of the nose from the odor of the roses.²⁰ In 1630, Sanctorious Santorio, a Venetian physician, described the case of a Venetian who experienced asthma on exposure to cats.¹⁴⁵

Concurrently, it became increasingly evident that skin also could be the site of "strange" developments in which it was affected solely or in association with nasal, bronchial, or ocular involvement. In the 17th century, Van Helmont, a Flemish professor of medicine and philosophy and an asthma sufferer himself, wrote of a canon with rhinitis and asthma who for "almost the entire summer . . . his entire body itched; white blebs were formed."⁸² In his 1713 pioneering work *Diseases of Workers*, Bernardino Ramazzini, an Italian professor of medicine at Padua and "father of occupational medicine," described sifters and measurers of grain who were affected multiply by grain dust and experienced cough, ocular inflammation, tearing, and intense itching of the skin similar to "nettlerash."¹³⁴ The 1796 classic illustrated text on cutaneous diseases by Robert Wilan, physician to the London Dispensary, documented numerous cases in which almonds, mushrooms, herrings, crabs, mussels, and lobsters were responsible for lip, facial, and tongue swellings, itching, and generalized urticaria and skin eruptions associated with throat constriction, cough, and gastrointestinal symptoms.²⁰⁶

According to anecdotal and historically relevant literature, such types of "strange" skin involvements also were known outside of medical practice. Britannicus, son of the first century's Roman emperor Claudius, was afflicted with tearing, facial swelling, and skin rash after mounting a horse.¹⁹² The 15th century English monarch Richard III, in a contrived plot, passed off his strawberry-induced skin eruption as a manifestation of witchcraft.^{108, 150}

Backed by three subsequent centuries of additional accounts of

similar experiments of nature, the stage was thus set for viewing skin as an appreciated tissue reflector of systemic sensitization and for exploiting its property as a potential investigational medium.

Shared reactivity of conjunctivae, nasal mucous membrane, and skin became understandable with recognition of their common derivations from ectoderm and certain microanatomic features: (1) rich capillary supply for immediate responsiveness to challenges manifested by vasodilation, increased blood flow, vessel permeability, and edema and (2) populations of mast cells in dermis, conjunctival epithelium, and sub- and intranasal epithelium associated with inflammatory reactions.

DETECTION OF HYPERSENSITIVITY: GENESIS OF SKIN TESTS

By anecdote, the earliest account of a skin test for idiosyncrasy dates back to 16th century Elizabethan England with the investigation of a complaint of intolerance to roses from one of Queen Elizabeth's ladies-in-waiting. A rose petal placed on her cheek during sleep that evoked a blister⁵² was perhaps the first designed single-blind study. In 1656, the similar raising of a 'blister' by egg applied to a patient's skin added credence to a French physician's investigation of food intolerance.¹⁹

Two centuries later the argument for pollen as the agent of hay fever⁶³—against the conjecture of summer heat and sunshine, odors, and emanations from hay, dust, ozone, and volatile benzoic acid from grasses—initiated the first approach to definitive testing for a verifiable cause. William Kirkman, a physician superintendent of an English asylum and a seasonal hay fever and asthma sufferer, undertook an out-of-season self-challenge in the winter of 1835. Sniffing a sample of blossoming sweet vernal grass in his greenhouse reproduced an attack of his summertime nasal symptoms and asthma.⁹¹ Thirty-seven years later, Morrill Wyman, founder of a Cambridge, Massachusetts medical school, conducted a controlled experiment involving direct testing at mucosal sites. In 1872, he gathered together a small group who deliberately sniffed summer-blossoming Roman wormwood, which provoked ocular, nasal, and asthmatic symptoms, to establish Ambrosia (ragweed) pollen as the cause of North American "autumnal catarrh."²¹⁰ The following year in England, Charles Blackley, a Manchester homeopathic physician and hay fever and asthma sufferer, employed additional approaches to original methods of testing. As his own subject in his home and office setting, he both deeply inhaled grass pollen specimens taken from his greenhouse and applied them to conjunctival, nasal, and buccal membranes, reproducing symptoms of ocular, nasal, and bronchial involvement.

Blackley also first demonstrated the capability of skin to serve as a test tissue. In a pioneering attempt at desensitization, he applied moistened pollen grains into abraded and scarified arm and leg areas with resultant induction of whealing.¹⁵ In retrospect, an earlier clue to the

skin test method could have been found in the 1850 classic textbook by Henry Hyde Salter, physician to London's Charing Cross Hospital. A pioneer in the study of asthma from which he also suffered, Salter described formation of wheals following cat scratches in patients who experienced asthma on exposure to cats.¹⁴⁴

After 30 years of neglected recognition of the significance of Blackley's innovative studies, a 1903 publication by William Dunbar in Hamburg, Germany identified and isolated an albuminous grass pollen component ("proteid") to provide a reagent for exhaustive studies and a reintroduction of the subject.⁵⁹ Meanwhile two seminal reports turned attention to a newly discovered aspect of hypersensitiveness. In 1902, Paul Portier and Charles Richet from the University of Paris reported the experimental induction of systemic shock reaction ("anaphylaxis") as an unexpected 'toxic' effect of immunization.¹²⁸ The following year at the Institut Pasteur in Lille, Maurice Arthus described a local anaphylactic counterpart—tissue injury induced by subcutaneous immunization and challenge—the "Arthus phenomenon."⁸ Influenced by attribution of a putative poisonous role for the immunogen in the decreasing recipient protective response, Dunbar considered pollen to be similarly toxic in its action as the anaphylactic agent of human hay fever. To identify specific offending plant and grass pollens and determine an individual's relative susceptibility—including his own—he prepared a toxin-antitoxin tissue diagnostic reagent (pollen 'proteid' + horse anti [pollen] serum). Reliance on evoked test reactions at ocular and intranasal test sites and precise quantitative calculations for determining theoretically exact therapeutic doses apparently did not prevent missing the practical mark. On receiving the first trial injection of his grass pollen extract, Dunbar developed a violent systemic reaction—the earliest in anecdotal records of pollen desensitization protocols.

In another innovative approach, Dunbar demonstrated that cat saliva alone contained the principal responsible for anaphylactic cat "idiosyncrasy." Positive reactions induced at scarified skin sites to applied cotton plugs saturated with cat saliva duplicated Blackley's experience with grasses. By cutaneous testing, Dunbar provided the first validation of an agent other than pollen to be responsible for the symptom pattern of hay fever.

In the subsequent development of skin testing methodology, attention was redirected to the "Koch phenomenon"⁹³—attenuation of evoked tissue lesions at the sites of subcutaneous injections of tubercle bacilli in tuberculous-infected guinea pigs. In an attempted therapeutic application, administration of a bacterial culture filtrate resulted in tubercle reactivation and severe systemic reactions. The procedure also proved that tuberculin-induced production of an inflammatory dermal lesion in tuberculous patients could serve as a diagnostic indicator of infection.

Revelation of the positive tuberculin test reaction opened a new skin test route for gaining insights into the pathogenesis of hypersensitiveness as: (1) a medium for the study of "delayed" hypersensitivity (48-hour reactions in contrast to the induced 15-minute, transient, erythema-wheal of "immediate" hypersensitivity), (2) a prototype for the

development of comparable skin test procedures for the study of infectious diseases caused by a variety of other microbial agents, (3) an additional method for the study of allergic-type disorders not relevant to or explained by anaphylactic (and later atopic-type) disorders, and (4) a supplement to tests for immediate whealing responses in uncovering information on phenomena common to infectious and immediate hypersensitivity processes.

As a modification of Koch's technique, Clemens von Pirquet at the Vienna Kinderklinik in 1907 replaced the subcutaneous injection of tuberculin with its application to a superficially scratched site.¹²⁶ Two years later, Smith reported the study of a patient who since childhood experienced a symptom complex of cough, itching, throat discomfort, hives, and giant swellings after eating buckwheat. Believing it to be a rare case of human buckwheat poisoning—previously known only in photosensitive cattle—Smith with Johns Hopkins' colleagues Thayer and Cole adapted the tuberculin test to their diagnostic study.¹⁵³ Buckwheat rubbed into scarified skin evoked a positive, immediate whealing reaction and a systemic anaphylactic response, which was the first recorded constitutional reaction induced by a skin test. A similar line of thought was pursued in 1910 with Schmidt's use of the von Pirquet cutaneous test to investigate children's reactivity to a commercial preparation of meat extract, egg white ("puro"), and trypsin-digested milk.¹⁴⁹

At about the same time, Leonard Noon and John Freeman, working in London's St. Mary's Hospital Inoculation Department, began their seminal work on the prophylactic treatment of hay fever in the persisting belief of grass pollen's putative role as a poison.^{73, 114} Like Blackley, they demonstrated immediate whealing in reactions to pollen samples applied to abraded skin sites. In their immunization protocols, however, positive scratch tests had only limited usefulness as markers of hypersensitivity. For determining the degree of sensitivity to grass immunogen, Noon and Freeman, like Dunbar, relied on conjunctival responses to ophthalmic challenges.^{70, 114}

In 1908, the tuberculin diagnostic procedure method was again modified. Mantoux introduced the intradermal method,¹⁰³ and Schick adapted its use to a test for susceptibility to diphtheria infection with reagent diphtheria toxin.¹⁴⁷ In reciprocal fashion, other investigators, whose studies centered on infection and immunity, found techniques that had been designed for diagnoses of hypersensitivity states adaptable to modifications of the tuberculin test. In 1907, Calmette developed a conjunctival test procedure,²⁵ and Moro described a percutaneous application technique (transdermic absorption through unbroken skin), which was, in principle, the method that emulated Jadassohn's diagnostic patch test for eczematous contact dermatitis.¹⁰⁹

By the second decade of the 20th century, methodology was thus available for varied skin test procedures to aid in studies of hypersensitivity phenomena and infectious processes. Particularly pertinent were investigations directed to inter-relationships of these processes, and the pathogenesis and diagnosis of their respective clinical manifestations and disorders.

IMMEDIATE HYPERSENSITIVENESS AND ATOPIC DISEASES: DEVELOPMENT OF SKIN TESTING METHODS

In their investigations of serum sickness, von Pirquet and Schick identified a circulating, humoral, precipitating antibody to normal horse serum and correlated its appearance with times of onset of symptoms after administration of antidiaphtherial and antistreptococcal antitoxins.¹²⁶ By contrast, Dunbar was unable to demonstrate a comparable circulating antibody as antigen-antibody complex in hay fever patients who manifested immediate conjunctival reactions to grass pollen.⁵⁹

In the absence of humoral, diagnostic, *in vitro* indications of the hay fever-asthma-eczema group, evaluations centered on observations of responsive tissues. The adequacy of conjunctival sac and nasal mucosa was offset, however, by procedure-induced discomfort and the limited number of test sites available for repeated challenge during a single testing period. Alternatively, large areas of skin surfaces on the arms, back, and thighs were found to readily lend themselves to simultaneous multiple site challenges.

The Scratch Test

Practical application of cutaneous testing for clinical hypersensitivity got underway in 1912 with a carefully controlled, in-depth investigative study by Schloss.¹⁴⁸ Applying von Pirquet's tuberculin-abrasion technique, Schloss correlated 5- to 15-minute erythematous whealing reactions with his interpretation of a child's history of rhinitis, asthma, and eczema as "idiosyncrasy" to egg, almond, and oats. Two years later Talbot, following up Schloss' method, reported 11 cases of childhood eczema and asthma similarly traced to dietary egg.¹⁶¹ Pivotal in adapting tuberculin testing to atopic disorders, Schloss introduced chemically extracted food fractions as skin test reagents. In another approach, Talbot rubbed egg white into intact skin according to Moro's percutaneous method. That imprecise definition of allergenic agents and mechanisms still lingered was reflected in Talbot's reference to his investigation of "egg poisoning."

During that concurrent period, Clowes initiated the earliest protocol for injection treatment of North American autumnal hay fever with injections of ragweed pollen extract. In so doing he moved Noon and Freeman's conjunctival method for pre-"immunization" testing away from conjunctival sac installation to the scratch technique.³¹

Extension of Schloss' work soon followed in studies of hypersensitivity disorders that correlated the: (1) identification of suspected airborne and food etiologic agents, (2) confirmation of observational leads and clinical impression by skin tests, and (3) preparation of testing reagents by laboratory procedures designed for extraction of reaction-inducing components of atopens. Shortly thereafter, definitive work was

begun in Boston medical centers on the relevance of positive skin test reactions to the clinical expressions of asthma and allergic disorders.³⁸

Coincident with Talbot's report in 1914, Goodale published the first in his series on hay fever, describing work done with extracts prepared at the Massachusetts General Hospital laboratory to determine by skin testing the biologic relationships between different plants.⁷⁶ Additionally, he furthered the use of the scratch test in studies of bacterial-derived proteids. Tests with diphtheria horse antitoxin, including direct application to the nasal mucosa, did not effect a definitive procedure for detection of susceptibility to serum sickness. Nevertheless, his work was directly applicable to defining anaphylactic reactivity in "horse asthma."⁷⁷

From an interest in serums and vaccines, Walker in 1917 turned his attention to studies of animal, vegetable, and pollen sources of protein.¹⁸⁵ A prolific flow of research reports followed.^{186, 191}

Through Walker's association with Wodehouse, a research chemist, the process for preparing dried, acetone-precipitated powders was developed and introduced into scratch testing procedures. These powdered reagents proved to be feasible and stable reagent alternatives to allergenic extract solutions.²⁰⁸ A variety of modifications followed, each with its own proponents,^{161, 178, 189} including: (1) abrasions created with a von Pirquet designed borer or by twists of a screwdriver blade, (2) precisely defined minute differences in sizes of scratches from 1/32 to 3/8 inches, and (3) differences in mediums of test reagents that included dried powders moistened with N/10 NaOH, rubbed-in paste, or drops of extract solution with added glycerin preservative. In all instances, the necessity of ensuring only superficial cutaneous penetrations was stressed. The possibility of absorption and access to open capillaries posed the risk of provoked constitutional reactions.^{95, 153}

Intracutaneous Test

Two years after Mantoux's modification of the tuberculin test (introduction of reagent into superficial skin layers by hypodermic syringe and needle), applicability of the intradermal/intracutaneous technique to the diagnosis of hypersensitivity was demonstrated. In 1910, Moss reported the use of samples of foreign sera to test for predictive contraindications to therapeutic administration of corresponding antisera.¹¹⁰ The technique was not used until Robert Cooke in 1916 summarized his 5-year study of the diagnosis and treatment of early (grasses) and late (ragweed) seasonal hay fever. The basis of his extensive series of patients differed from those of Noon and Freeman^{70, 114} in three specific aspects: (1) recognition that causative pollens acted as sensitizing, not poisonous, agents, (2) awareness that injection treatments were designed to desensitize (or hyposensitize) rather than produce protective (antitoxic) immunity, and (3) preference for his own adaptation of intradermal over ophthalmic and cutaneous (scratch) test techniques.⁴⁰

Adoption and broad use of the technique by Cooke and his associates at the first multistaff allergy clinic, opened at New York Hospital, was made possible by Coca's contributions from his affiliated hospital's first laboratory of applied immunology. Delivering sterile, stable, nonirritant, atopen-specific testing solutions to the clinic staff required Coca to develop individualized procedures of extraction, preservation, and assay for dealing with differences in chemical character and physical properties of a wide range of perennial inhalants, foods, and pollens. Undertaking the preparation of extracts that were newly introduced into clinical medicine and adapted for unique purpose and use presented the need to virtually develop a new area of chemistry.

Minimizing the loss of allergenicity by preserving stability and preventing alteration of extracted proteins and at the same time ensuring the action of chemical bactericidal additives and removal of irritants and irrelevant source components were among the requirements for developing these procedures.³³ In later years, other problems surfaced from the incorporation of antibacterial merthiolate in extracts administered to mercury sensitive subjects¹³⁷ and the contamination by microscopic crysolite-asbestos fibers in extract solutions that passed through microbial filtering Seitz pads¹¹⁸ or were delivered from Cooke glass syringes constructed with asbestos thread wound around tips of steel plungers.¹³ Though the latter ensured tight fits of interchangeable syringe plungers and barrels, injections of asbestos-containing skin test extracts presented a number of serious considerations, including delivery of asbestos-absorbed extraneous antigenic proteins, migration of asbestos fibers from skin test sites to other tissues, unknown potential carcinogenic effects of asbestos, and presence of leached magnesium.

The need to keep stacks of freshly prepared extracts under adequate refrigeration in the era of ice chests and to maintain large stocks of resterilizable glass syringes and steel needles in the days before the availability of disposable units, could not be bypassed. Duke practiced a short cut that he described in 1926 as the "one syringe method."⁵⁵ After completion of an intracutaneous test, the single-used syringe and needle were washed thoroughly and sequentially in each of three vessels containing sterile salt solution. With each passage, he found residual extract greatly diminished, and by the third rinse, any remaining trace in the syringe was too dilute to interfere with reuse on subsequent tests. Further, the "third water (was) not contaminated sufficiently" to preclude repeated and continued use of the same three washing solutions.

Of questionable advance, a later method, which in retrospect, introduced a new set of faults, also gave priority to practicality. It became common practice to use the same extract-loaded syringes to test more than one patient during any clinical session or office period. Heat-sterilized glass syringes and attached steel needles were filled with sufficient quantities of extracts to last during a several hour, multipatient testing session and were loaded in holding trays. Needles were changed sometimes but more often were only wiped with alcohol between tests

done on patients whose Wasserman-negative states and negative history for malaria were ensured. Serum-transmitted viral hepatitis was an unknown during those early years. (Observation based on author's [SGC] remembrance of first witnessing routine allergen skin testing in World War II Army hospitals and early postwar Veterans Administration clinics.)

Additionally, there was the safety factor that favored the scratch test advantages of weaker challenges, slower and diminished absorption, and better control that allowed for prompt removal of applied reagent if a danger signal presented. A greater risk of anaphylactic constitutional reactions was associated with intracutaneously placed allergenic extracts. A noted variant, and often forerunner of a systemic reaction, was ascending severe itching and tenderness of axillary glands that represented lymphangitic pathway of a hypersensitivity manifestation extending upward from test sites on the forearm or medial aspects of the arm.⁴⁶ A constitutional anaphylactic reaction to fish-derived glue, responsible for the first, skin test evoked fatality was reported by Cooke in 1922.⁴¹ Yet the Cooke group stood firm in championing intracutaneous testing; to them it offered the advantage of greater sensitivity, quicker performance to completion of the endpoint response, direct observation unobstructed by any overlying coating, less masking and residual scarring at test sites, and availability of identical extract solution for dual purposes of testing and injection treatments.²²

Walker's comparison of the two techniques that favored the scratch method pointed out that intradermal test results: (1) did not always separate structurally close-related proteins and, therefore, could be less specific, 2) because of greater sensitivity could be disadvantageous by diminishing the value of the test result as an index to treatment, and (3) were affected variably by injection treatments resulting in erratic changes in degrees of positivity of responses.¹⁸⁹ In response, Walker's study was faulted by Brown for using too large volumes.²² A later demonstration by a member of Cooke's clinic staff that showed that scratch tests employing 50 to 100 times stronger pollen solutions did not miss any positive reactions elicited by intracutaneous tests⁷⁰ apparently did not change (published) opinions or practices of the group.

When in doubt of the determination of sensitization, Cooke's definitive statement allowed for an alternative by use of the ophthalmic test.⁴² There remained, however, two particular circumstances for which the scratch test did not have a substitute: (1) when diagnostic study favored skin testing with whole pollen grains, and (2) when investigating urticarial edema of the oral mucosa, only fresh fruit and vegetable juices could meet the requirements for dependable test allergens.¹⁶⁸

P-K Test: Indirect Method

Although the scratch and intracutaneous skin test methods found increasing use and acceptance as valid indicators of specific atopic

agents, the exact pathophysiologic mechanism responsible for inducing immediate wheal-flare reaction remained undefined. Among popular advanced, immune-related concepts were the actions of tissue cell-attached antibodies and sensitization, the results of putative "lytic immunity" (i.e., effected by development of special ferments that attack particular proteins and render their split products poisonous).¹⁷⁷ The breakthrough in understanding came in 1921 with an experiment by Prausnitz (P) and Kustner (K). By reciprocal injection of a sample of each investigator's serum to the other and intracutaneously testing the recipient sites, they demonstrated successful passive transfer of K's fish sensitivity to P's non-fish serum. P's pollen sensitivity was not transferred.¹²⁹

In 1925, Coca and Grove—by extended study of the P-K reaction—verified the role of the serum factor responsible for positive skin test reactions with corresponding allergens.³⁴ They named these antibodies atopic reagins (Greek for reactive [substance]). Demonstratable only by the P-K method (unlike identifiable *in vitro* antibodies), reagins were later isolated, chemically characterized, defined as a gammaglobulin fraction, and named immunoglobulin E (IgE).

Appreciating the specificity of transferable action and demonstration of P-K reaginic antibody, Matthew Walzer initiated a series of studies on its skin-sensitizing character that led to its adaptation as an indirect method of skin testing.^{193, 196} The technique was found to be especially useful when the direct method was deemed impossible, impractical, or inadvisable (e.g., in the presence of eczematous, ichthyotic, and infectious skin disorders, risk of inducing constitutional reactions, urticaria, dermatographism, or hyperirritability of skin [particularly characteristic of infants] that produced false-positive reactions; and various reasons for inability of the patient to get to a reliable facility for direct testing). In such circumstances, the skin of a volunteer (a normal substitute who received intracutaneously transferred samples of serum taken from a patient under study) became passively reactive to the subject's causative allergen. Transferred sensitization was evanescent and localized to the demarcated sites which were then tested after a delay of 12 to 96 hours to allow for subsidence of initial, procedure-induced, nonspecific skin inflammation. Walzer also found the technique to be of particular value in the search for food allergens. Challenges with suspected and controlled foods could be affected by test feedings through which gastrointestinal absorption and circulatory delivery bypassed the necessity of intracutaneous needle challenge of each passively transferred site.

Modifications of the P-K test method allowed for investigation of paradoxical instances of clinically demonstrable symptoms with negative skin tests to specific foods. In such circumstances, the causative allergen was viewed as a secondary derivative (i.e., not acting in its primary state as ingested but rather as a split product of digestion or metabolism). In reversal of the P-K procedure, Abraham Walzer demonstrated that ingestion of test food, later followed by intracutaneous transfer of test serum,

elicited a spontaneous reaction at the test site.¹⁹⁴ In a further variation named autopassive transfer, intracutaneous sites of the patient's own (autogenous) transferred serum reacted to allergens introduced at remote areas.⁴⁹

In another application of the P-K method, the Cooke group developed a novel procedure for studying serologic changes effected by immunotherapy. Their observation, that the addition of serum recovered from a hay fever patient to a pollen test extract required a higher pollen concentration to evoke a positive skin reaction, provided the basis for assessment. Through this technique, relative changes in the allergen-neutralizing capacity of serum samples could be followed and evaluated.⁴³

The P-K procedure was especially well-accepted by parents who wished to spare their infants and young children with asthma and allergic problems the emotional and physical trauma of direct testing. The feasibility of shipping sera from collection points overcame obstacles of geographic separation and distances between patient and physician. Collection of serum samples for study by clinical investigators who required supplies of specific reaginic character also enhanced cooperative research studies.

The complicating effect of constitutional lighting up of multiple sites by absorbed, strongly reactive test allergens that entered into and were distributed by the circulating blood stream required paying special attention to details of technique. In the unfolding course of events, however, it did not serve as the critical objection to allowing a continued role for indirect testing. Whatever value the P-K method may have had in diagnostic study of allergic disease was overshadowed in the mid-1960s by discovery of the hepatitis B virus and its identification as a bloodborne, serum-transmissible infectious agent.

Among the aids to relevant research endeavors that were necessarily terminated was the centralized resource of a serum bank established by Bernard Siegel and M. Walzer at the Jewish Hospital of Brooklyn. A pertinent function of the facility had focused on the collection, storage, and distribution of serum samples of reaginic antibodies with correspondence to atopens of special character. A primary resource for collaborative investigation of protocols developed by the Research Council of the American Academy of Allergy thus was lost.³⁷

Testing for Atopens: Cutaneous Reactivity, Factors, and Influences

Progress in the development and refinement of clinically applicable aids in diagnoses of atopic disorders and identification of etiologic allergens generated investigations centered on various aspects of skin testing as a method. Studies were designed to contribute to the understanding of mechanisms that both effected and affected wheal-flare endpoint results.

The fact that allergen extracts could not be expected to evoke invariable all-or-nothing responses came to light in 1922 with Cooke's demonstration of nonspecific, progressive diminution of whealing capability at positive reacting, repetitiously tested sites.⁴¹ Explanation of the development of exhaustion was found in the work of Sir Thomas Lewis on the physiology of wheal-flare phenomena.⁹⁷ In a series of reports from 1924 to 1927, Lewis described the identical progression of varied whealing responses from initial, cutaneous, microvessel, capillary dilatation to reflex arteriolar dilatation (flare) to increased permeability, serum transudation, and ultimately demarcated and localized edema. Whether effected by injury or injection of histamine in normal individuals or by specific allergens in hypersensitive subjects, cutaneous endpoints were identical. In each instance, depletion of a common conceptualized "H-substance" mediator resulted in refractoriness to same site and repeated challenges (and skin tests).⁹⁷

The flow of studies that followed demonstrated a variety of local and systemic influences on expressions of histamine and skin test responses. In particular, inhibition and diminution of whealing were characterized by or associated with circulatory impairment,¹⁵⁶ dehydration,¹²² skin injury and stroking,⁹⁸ temperature changes by immersion in cold or hot water,⁹⁷ or inflammation, edema, edematous skin changes of thyroid disease,¹²³ acute inflammatory dermatitis (e.g., erythema multiforme, pemphigus), chronic dermatoses (e.g., psoriasis, lichen planus, lupus erythematosus), skin atrophy, and thickening (e.g., ichthyosis).¹⁹⁵

Other evaluated variable influences included: (1) specific areas of testing (back, thigh, and forearm manifested a decreasing order of sensitivity),⁵ (2) concentration⁷⁰ and quantity⁹⁴ of test extract, (3) degree of hypersensitiveness (i.e., serum reagin content),⁹⁶ (4) diminishing effect of allergen injection treatments (hyposensitization),¹⁸⁷ and skin irritability (i.e., dermatographic response),⁹⁷ (5) proximity of adjacent test sites as source of induced reflex erythema,⁹² and (6) positive skin tests as prognostic forerunners of clinical sensitivity or representations of past atopic diseases.⁹ There was essentially no need to consider the concurrent administration of symptom-relieving medications (i.e., ephedrine, epinephrine, theophylline, stramonium, atropine, lobelia, nitrates) that did not have any appreciable effects on skin test responses. With the development of antihistaminic agents in 1937, their pharmacologic inhibition of whealing responses introduced a confounding factor. Although creating a problem, this property at the same time was turned into advantage; pre-skin test administration curtailment of dermatographic responses allowed for interpretation of valid cutaneous reactions to allergens.⁶⁸

Delayed Reactions

In developing the scratch test method, Walker and Adkinson in 1917 noted the occasional development of a second type of response that appeared after 24 hours at initially "practically negative" test sites.¹⁸⁹

Describing these "delayed positive reactions" as "hot, very red, slightly elevated . . . resemble a mild infection (but sterile)" that disappeared over the next 2 days, they equated the significance of delayed responses with the responses of immediate hypersensitivity. Other subsequently reported experiences and interpretations, however, were not in agreement with Walker's observations. Conceptually, immediate wheal-erythema responses replaced by or progressing to delayed reactions possibly might be viewed as a continuum of evolving manifestations of the same immune process. Evidence, however, could not be found for a reaginic antibody mechanism to explain the development of a delayed reaction in the absence of a preceding evanescent, immediate hypersensitivity test response. Particularly was this the case with food and pollen allergens in contrast to microbial antigenic products in tuberculin-type sensitization and test reactions.

M. Walzer's extensive series of studies by the P-K indirect method initiated in 1925¹⁹⁶ were helpful in clarifying the finding that delayed reactions were equally and especially frequent in normal and atopic subjects alike, and were never found apart from identical reactions at control sites. They could not be considered of any interpretable significance, especially in the absence of a related clinical history.

Warren Vaughan's investigation of controversial interpretations differentiated the roles of the eliciting methods. His evaluation centered on the point that, in contrast to scratch test reagent powders, intracutaneously introduced allergen extracts often contained nonrelevant irritating materials that could not be removed during preparation processes. Extending that observation, he noted that the less frequent, scratch test-induced, delayed reactions were more likely to be demonstrated at 4 to 6 hours in disorders characterized by chronicity. This especially was the case in eczema attributed to an almost daily ingestion of allergenic food.¹⁸⁵ The concept that the mechanism for induction of immediate positive-whealing reactivity could be suppressed (and perhaps even replaced by delayed responses) was suggested by a pertinent observation. Following an acute asthmatic attack or in instances of frequent contact with an allergen by natural exposure or by repetitious hyposensitization treatment injections, positive immediate reactions to the triggering adverse agents were diminished greatly, even to a "nearly negative" degree. By viewing delayed reactions as a counterpart of the Arthus reaction, Vaughan believed they represented a valid hypersensitivity manifestation. Although milder in degree, delayed skin test reactions and their development were comparable with the local, subcutaneous, inflammatory reactions occurring within a few hours after repeated injections of foreign species antitoxins in patients who developed sensitization to the animal-source serum proteins.¹²⁴ In the absence of a search for associated, circulating, precipitating antibodies to reflect the presence of antigen-antibody complexes, or in the absence of biopsies to differentiate predominantly edematous and infiltrative cellular qualities that are characteristic of immediate wheal-flare and delayed reactions (respectively), the concept remained a hypothesis.

Adding to the validity of a two-phase immune process was the experimental study of Simon and Rackemann.¹⁵² Human subjects receiving series of intracutaneously injected guinea pig serum produced both immediate reactions caused by production of skin-sensitizing reagins and, 18 to 24 hours later, red, swollen, delayed reactions. These findings, however, could have little if any correlation with Rackemann's earlier consideration that uncommonly occurring, delayed reactions were "merely confirmatory" of results of immediate tests.¹³⁰ Unlike food, pollen, and perennial inhalant allergens whose immune reactivity related to those persons affected through genetic predisposition, foreign sera evoked antigen-antibody complex phenomena responsible for delayed (Arthus type) reactions in atopic and nonatopic individuals.

Cooke believed that several different mechanisms were involved in the production of the endpoint of delayed reactions and thus did not share the commonality of an immunologic entity. From one mechanistic aspect, however, he reported that delayed intracutaneous reactions to certain food test extracts were valid manifestations of immediate hypersensitivity. The delayed expression was attributed to the time required for digestion and cleavage of the protein from which the responsible allergen—as an altered product—was derived.⁴⁹

The description and definition of late phase cutaneous test responses (LPR) in 1973⁵⁸ is relevant to this point. Had that knowledge been uncovered some 40 years earlier, perhaps Walzer, Cooke, Vaughan, and Rackemann might have given different interpretations to the positive delayed reactions noted within the initial 4- to 6-hour test period. Whether immediate hypersensitivity LPR or accelerated (antigen-antibody complex mediated) Arthus phenomena, the correct interpretation remains a matter for retrospective speculation.

SKIN TEST MODIFICATIONS AND ADAPTATIONS

Prick-Puncture Test

Scratch and intracutaneous techniques remained the primary methods of testing for validation of or search for causative atopens for some 60 years after their introductions into clinical medicine. Ultimately proponents of both techniques gave way to adoption of the prick-puncture technique once reports of its surpassing advantages began to be explored and appreciated in the late 1970s.

The skin borer designed and used by Pirquet in his 1907 modification of Koch's subcutaneous tuberculin procedure¹²⁶ had been visualized as the prototype instrumentation for prick-puncture testing. Application of the concept, however, can be traced back to earlier studies of experimental urticaria. To induce whealing, the point of a fine open-capillary tube dipped into solutions of morphine, atropine, peptone, or paraphenylamine¹²¹ (the earliest identified chemical agents of nonspecific

or nonhypersensitivity mediated whealing) were thrust into (but not through) the skin.

When Eppinger discovered the scarification-induced urticarial reaction to histamine in 1913,⁶⁵ the method became the medium of studies using the puncture technique. Four years later, Sollman and Pilcher described an innovative approach in which skin sites were rubbed with dried, stiff, legume seed-pod hairs of the tropical mucana plant followed by application of histamine solution.¹⁵⁴ Further technical variations that led to development of the definitive prick-puncture technique originated with the vascular studies of Lewis and Grant. For induction of wheals to investigate capillary circulatory dynamics, they first experimented with a mucana-like technique in which spun glass was substituted for the plant hairs. In a second procedure that they introduced, histamine was forced into skin through drawn fine points of capillary tubing.⁹⁹ In subsequent comparative studies of histamine-induced and anaphylactic skin reactions, their most satisfactory procedure evolved from piercing drops of herring and smelt extracts into the skin of a patient who experienced oral swelling and itching, urticaria, and asthma on eating fish.⁹⁹

During the next 50 years of continuing predominance of scratch and intracutaneous methods, prick-puncture testing went unheeded in the United States. Squire's intervening 1950 study of horse dandruff and serum antigens¹⁵⁵ briefly recalled Lewis' earlier innovation. Ultimately, Pepys' seminal work on bronchopulmonary aspergillus¹⁰⁵ reintroduced the technique. Largely as a result of Pepys' continuing popularization in the 1970s,¹¹⁹ the advantages of prick-puncture (specificity with minimization of trauma, discomfort and irritant effects) were increasingly appreciated and gained widespread adoption.

Serial Dilution–Titration Test

In 1935, French Hansel introduced a quantitative modification of the intracutaneous method. Each positive-reacting extract was tested in decreasing concentration until the weakest dilution that elicited a positive whealing response was reached.⁸⁰ According to Hansel's protocol, each individually tailored starting dose of treatment extract—for purposes of safety and believed efficacy—was equated with and determined by the correspondingly demonstrated endpoint test reaction.

Fifteen years later, Rinkel reported his modification of the serial titration method based on an arbitrary set of calculations using multiples of endpoint figures. The Rinkel proposed formula for establishing optimal doses, at which symptoms could be expected to be controlled by immunotherapy,¹³⁹ lacked verification by controlled investigation. Application of the concept thus did not achieve the status of an accepted standard modality in mainstream clinical practice.

In later years, the Rinkel method was integrated into a further unconventional and controversial procedure. Intracutaneous, serial end-

point titration testing was applied to putative determination of "provocation" and "neutralization" doses in a scheme of diagnostic criteria. Initially described for food allergy,¹⁴⁰ extension of the procedure to inhalant agents and environmental chemicals followed. Again there was an absence of validating data generated through controlled investigation; provocation-neutralization skin test protocols remained unproven.

Percutaneous Test

Bypassing the need for infiltration by hypodermic needle, the Moro test inunction method (gently rubbing tuberculin incorporated into a bland ointment into an intact skin site) proved well suited for demonstrating a test response indicative of tuberculous infection. However, its ability to evoke a follicular papular reaction of the delayed tuberculin type was not applicable for use with allergen extracts to effect the reagin-mediated, vascular, whealing immediate hypersensitivity.

The property of large molecular size that endowed protein allergens with antigenicity exceeded the critical mass that enabled molecular penetration of the epidermis. Exceptions to surface impermeability of food and inhalant source allergens could be seen in circumstances of urticarial lesions appearing at sites of atopen contact in the skin of infants and young children with atopic dermatitis. The role of immediate hypersensitivity to eruptions of contact urticaria on the hands of adults was demonstrated by positive scratch tests with certain handled raw foods but was not evoked by intracutaneous tests with allergen extracts.¹⁰⁹ Observations that suggested the transepidermal route of testing might be possible led to searches for vehicles to deliver incorporated allergen extracts to vascular cutis. Percutaneous penetration of powdered protein preparations was enabled by the development of nondenaturing solvents. The most effective vehicle of this character consisted of a mixture of alkyl-benzene sodium sulfate, antipyrine, and propylene glycol.⁸³ The advantage of this painless atraumatic method was weighed against the occasional occurrence of false-negative test reactions.⁸⁴ From another viewpoint, false-positive reactions seen in dermatographic skin could be differentiated by the atypical nature of the wheals.

Electrophoresis

In 1938, Abrahamson reported finding galvanic current to be efficacious in driving (charged ions) extracted grass and ragweed pollens and histamine through pores of the skin.² Positive test responses were manifested within 5 to 10 minutes of almost imperceptible electrical currents. Induced wheals were comparable with those produced by conventional needle techniques. Additionally, iontophoresis offered the advantage of an atraumatic procedure leaving skin test sites intact. Abrahamson then took his work one step further using the electropho-

retic method to replace hypodermic (needle) administration of pollen extracts in hyposensitization-injection treatment programs.³

The electrophoretic technique remained a scientific research tool rather than finding a place in allergen skin testing and immunotherapy. Nevertheless, its demonstrated ability to transport antigens through the dermis (and putatively beyond through systemic channels) provided the medium for a unique and imaginative experiment in skin testing by A. Walzer and Golan that was described in a 1945 published report¹⁹⁷ and demonstrated as a program presentation at the preceding first annual meeting of the American Academy of Allergy. Three nonatopic, normal individuals were joined by clasped hands. Electrophoretic introduction of antigens into the skin of the arm of the subject at one end was followed by putative transport of the antigens through all three linked by the same electrical circuit. The result was immediate positive whealing responses at sites of passive sensitization with corresponding reaginic sera in the arm of the third joined subject at the opposite end. As an intriguing, appreciated, and puzzling offering never repeated or at least reported by other investigators, the mechanism remained unexplained.

Patch-Abrasion Test

Tucker and Thomas' suggestion of a combination of scratch and patch test techniques was validated in instances of atopic (eczematous) dermatitis and urticaria in which intracutaneous scratch and patch tests were individually negative.¹⁶⁶ Adhesive covering placed over scratch test sites to keep testing material in place for prolonged contact facilitated development of positive responses. The delayed type of evoked reactions, however, limited the value and interpretation of this procedure.

Skin Window

Realistic appreciation of the nature of skin test reactions as diagnostic mirrors of the atopic diseases under study enforced Cooke's proffered tenet: "With these tests one must duplicate the essential histologic characteristics of the existing lesion."⁴⁸ Microscopic definition had established the pictures of (1) extravasation of fluid and some leukocytes through walls of dilated, damaged vasculature of immediate hypersensitivity⁹⁷ with eosinophils identified hours after fading of the urticarial wheal and (2) cellular predominance of leukocytes (polymorphonuclear, eosinophilic, and mononuclear) with edema and disruption of connective tissue fibers in the papular delayed reaction.⁸ Although microscopic examination of tissue response specimens would have satisfied Cooke's critique, the impracticality of performing routine diagnostic biopsies was self evident. For investigative pursuits, study of sequentially timed, fixed

tissue samples provided only inferential information on the dynamics of hypersensitivity responses until the introduction of the skin window.

In 1947, Rebuck described a method for studying the evolution of cellular migrations to inflammatory skin test sites.¹³⁵ Stepwise a small area of skin was abraded, a strip of epidermis was removed gently, a test agent was applied, and a series of sequentially placed, overlaying glass or plastic cover slips were removed, stained, and examined microscopically for identification of cell types found in the adherent exudate. Extended study by Rebuck generated information on leukocyte function in several diseases and responses to a variety of inducing agents.¹³⁶ The value of skin window techniques as a research tool was extended to the study of allergens and cytologic aspects of hypersensitivity.^{60, 61} A later modification replaced the cover slip with a chamber to collect transudates for cytologic study.⁶⁰

SKIN TEST ISSUES: CLINICAL AND PROFESSIONAL

In the early years of evolving new information on hypersensitivity, expressions of clinically relevant concerns and differences were limited to the small number of physicians beginning to center their interests on asthma and the newly defined group of atopic disorders. For those physicians giving special attention to the application of descriptive phenomena or development of diagnostic methods, there were few points of contention. Primarily, the major question discussed was the choice and acceptance of either scratch or intracutaneous testing. Arguments centered on the most reliable and valid method from standpoints of sensitivity, reproducibility, and relevance to the patient's condition under study. Within both pioneering groups—the Cooke circle of intracutaneous champions and the proponents of the scratch technique advanced by Goodale and Walker—were shared concerns. Their questions related to requirements and criteria for chemical extraction and assay of test materials, interpretation of delayed reactions, and a place for bacterial fractions in diagnostic evaluation of asthmatic and atopic patients.

It was not long, however, before dissemination of information, through literature reports, papers given at meetings, and the resource of newly established clinics, reached the medical community at large and generated new concerns. Unexpectedly, a well-intended mission of the new national professional societies—the Eastern Society for the Study of Asthma and Allied Conditions (SSAAC) and Western Association for the Study of Asthma (ASA)—went beyond the intended limited objective of creating awareness of allergy's genesis and its development as an emerging segment of medicine.

Inadvertently, easy accessibility to the tools for conducting skin tests was put into the hands of nonspecializing practitioners. Many were inadequately prepared to understand and apply the basic principles of critical indications and proper usage in patient care. Consequent opportunities for abuse or deviation from diagnostic and state-of-the-

art treatment, beyond the control of clinical experts, were decried in presidential addresses given at SSAAC's national meetings:

1931

"[Needs exist for] proper materials to do the work. At the present time there are as many different solutions and powders available as there are manufactures. These are not interchangeable . . . Until uniform solutions and methods can be established, the present chaotic state of individual effort will continue [for] education of the general practitioner. He should be taught that his unskilled efforts to utilize the expensive materials sold him by detail men are not likely to be of benefit to his patient or of profit to himself. He should be told the limitations . . . education of the public itself. There is a feeling today that skin tests are much like a Wasserman or a urine analysis and can be performed by anyone with a small outlay for equipment. So when the family physician with a few old food powder scratches their skin, they are under the impression that they have had the advantages of the latest that science has to offer. The need of special training in the technic and the interpretation of the skin tests . . . should be stressed."³⁰

1933

"We must combat the general attitude (which seems to be gradually gaining ground) that allergy testing consists of scratching the skin with a few food tests and putting the patient on a weird and impracticable diet which usually accomplishes no result. We should be frank in admitting that skin tests are of little value where the clinical reaction is delayed but equally insistent that they be done where the clinical reaction is immediate and we may expect them to be of the utmost importance. The reading of these tests and their interpretation should be done by a competent allergist."¹⁷⁴

1943

"We permitted the entrance into the field of some men who took the attitude that allergy consisted of needles, syringes, and a patient's extremity; men who failed to appreciate the fact that to know allergy one must first know medicine."²⁸

The comment referring to education of the public and practitioner could well have pertained to the language of a newspaper account in the *Boston Globe* (Fig. 1) that featured the work of Goodale's clinic⁷³: "Have you heard of the 'skin test'? It's one of the strangest new stunts discovered by the medical profession . . . Doctors scratch your arm, rub in a little powder, and tell you that if you stop eating eggs you will have no more headaches." (One might have questioned the reporter's intent in using the term *stunt* whether it was meant to be praiseworthy or otherwise. The reader's reaction to this description could have been one of uncertainty, perhaps giving reason to be mislead, fairly or unfairly, by casting a newly announced and unfamiliar medical procedure in the light of a theatrical or attention-gaining feat.)

DOCTORS TRY NEW DIAGNOSIS STUNT

Rubbing a Powder Into a Cut on Your Arm Tells You How to Cure a Headache by Not Eating Chicken or Avoid Hay Fever by Shunning Horses

Have you heard of the "skin test?" It's one of the strangest of the many new stunts discovered by the medical profession in their fight against human ills.

Right here in Boston experiments have been tried which attracted attention throughout the medical world.

Doctors scratch your arm, rub in a little powder and promptly tell you that if you stop eating eggs you will have no more headaches or that if you keep away from cows your asthma will disappear.

It is a new theory, pretty well known in medical circles; but the great bulk of us outsiders are just getting word of it.

Sounds Like Meedloine Man's Charm

Probably the oddness of the thing has much to do with the popular interest. It's hard for nonmedical folks to understand why scratching a man's arm with a little knife and working a tiny quantity of white powder into the cut should enable the doctor to decide whether or not the patient can eat chicken or if he had better give up horse-back riding.

But that, so the users say, is exactly what the test does do. And there are scores of people in Massachusetts today, and perhaps thousands throughout the country, who testify to its efficiency.

Most amazing results are reported: headaches of 30 years' standing cured by following a diet worked out through the test, serious heart trouble permanently relieved, hay-fever prevented year after year, asthmatic conditions corrected, stubborn indigestion made a thing of the past, skin troubles helped.

It sounds as mysterious as the charms of the ancient medicine men. Even the doctors who have used it most frequently and successfully don't know exactly the "why" of it all. But they do know



DR. J. A. TURNBULL

Figure 1. Newspaper feature article on skin tests related to Joseph Goodale and John Turnbull's work on hay fever and anaphylaxis (allergy) in the Massachusetts General Hospital Throat Clinic. Courtesy Saul Malkiel, MD, PhD and the Boston Globe, exact date of publication undetermined, circa 1916–1920.

Illustration continued on opposite page

A foremost textbook of the day also did not refrain from adding comment to its teaching dicta: "Skin testing, aimless, excessive, and misunderstood, had done more than its share in bringing allergy into disrepute."⁴⁵

The imperceptive practice of testing with excessive numbers of

14 Cuts on Each Arm

"These powders contain the proteins of various foods and animal extracts such as hornhair, the feathers of chickens and similar substances," explained Dr. J. A. Turnbull of Boston to a Globe reporter.

Dr. Turnbull is one of the champions of the skin test, and back in 1914 started a clinic for this work at the Massachusetts General Hospital. The clinic is now under the direction of Dr. Francis M. Rackemann of Boston.

Dr. Turnbull described the complicated method of preparing the powders and how they are introduced into the tissues. The forearm is usually chosen, he said, because it is more convenient and less sensitive than other parts of the body. But the leg or any other part can be substituted.

Tiny drops of a weak solution of an alkali are placed at intervals on the arm. The first drop is called the "control," that is, it is used to indicate how the skin reacts to a slight abrasion. No protein is used for this. A small, very sharp knife is used to cut the skin very lightly.

This process is repeated on the second drop, after a small amount of the powder has been dissolved in it, and the solution is gently rubbed into the tiny cut.

The process is repeated until some time as many as 14 incisions have been made on each arm. Since each powder represents one protein this means that tests have been made for 48 different sorts of food, or half the total number required to ascertain the proper diet. Each vegetable, meat or fruit is tested.

When Chicken is a Poison

Now, for example, the protein of chicken may have been used for the first drop. If chicken, as a food, agrees with the patient nothing will happen. If it does not agree with him, a redness or swelling, or both, will appear on the abrasion, varying in degree in proportion to the extent to which it is bad for the patient. The swelling or redness disappears in from half an hour to an hour. The next day the patient returns for the remaining half of the tests, after which a diet can be worked out.

"In cases of indigestion," said Dr. Turnbull, "the test gives us scientific means of ascertaining the exact cause of the trouble. There are some persons who may be sensitive to a certain food but are able to carry it without showing marked symptoms, until a time when they are weakened by worry, illness or exhaustion.

"When it is found that a certain food does not agree with the patient the best thing is to eliminate it entirely. If the protein of chicken is harmful to the patient it will be quite as harmful in

chicken broth as in roast chicken. It should be given up entirely.

"The alternative is the injection of small but gradually increasing doses of the protein until the patient is what we call 'desensitized.' In some cases this proven very satisfactory, in others it is not. In almost all cases it is better simply to stop eating the objectionable protein.

Pea Soup the Cause

"As for results—4 men of 33 had been suffering from headaches for nearly 20 years. He had tried every method suggested, had his eyes, teeth, sinuses and general condition examined without results.

"After two weeks on a diet, during which time he was free from headaches, he complained of a bad headache. Investigation showed that he had broken his diet by eating a little pea soup on the same day. Peas were one of the forbidden foods.

"He found by experience that as long as he stayed on the diet that was long trouble, but that if he gave it up the headaches returned.

"Some people are affected very quickly by even the smallest quantity of the food to which they are sensitive. With others, symptoms do not appear until there has been an accumulation lasting over several days.

"In many cases the food proteins cause symptoms of head colds, or other diseases. There was a woman whose case was diagnosed as asthma, persons who were relieved by adopting the diet in the test.

But the proteins of foods are not the only causes of trouble which this skin test aims to eliminate. Every hayfever victim knows that the pollen of certain plants, notably ragweed, are the cause of his misery.

There are other things less obvious which come within the scope of the test. Even face powder and perfumes have been found to cause occasional cases of asthma.

As to Hay Fever

"Patients with asthma," said Dr. Turnbull, "attribute attacks to dust, dandruff, smoke, dampness, cold air, east winds, changeable weather, excitement, indigestion, nervousness and cold. These are all secondary features, acting on a sensitive respiratory tract, which has a harmful protein. After removal of the cause and suitable treatment these factors fail to produce attacks and the disturbances are relieved.

"In cases of hay fever, due to the pollen of willow, alder, oak, birch, maple, apple, chestnut, grasses, rose, ragweed, golden-rods, holly, sunflower, can all be treated with pollen protein to which the patient is sensitive.

There are also asthmas brought about by the inhalation of the protein of horse dandruff, dog, cat, rabbit and guinea-pig dandruff, chicken feathers, feathers from various birds and fur-bearing animals. Some are rubbed by the horse dust in the streets.

"The method in these cases is to inhale

in the food protein case. Concentrated solutions of the proteins of the substance are rubbed into the cut and the reaction studied. The essence of horse hair or hayseed dust might be scratched into the skin.

"In some cases, such as asthmatic conditions produced by a feather pillow, the patient is simply told to avoid contact entirely.

"In others, like the hay fever mentioned above, a treatment is given consisting of injections of the pollen protein until the patient is desensitized.

"In the hayfever cases, 10 or 12 injections, a few weeks before the season of the plant whose pollen causes the fever, will generally give relief or temporary immunity."

No Panacea Claimed

"It is not a panacea," the physician declared. "We don't cure all cases; but it is a step forward in a scientific direction."

"Why is it that injecting something into the arm will tell whether the same thing was in the food which you eat?" asked the reporter.

"That is what we don't exactly know yet," replied Dr. Turnbull. "I have a theory that when the powder comes into contact with the tissues and produces irritation it means that there is in those tissues the presence of certain materials to neutralize the poisonous part of the food. Another possibility is that in the tissues there is a certain ferment which breaks the food up into parts which are poisonous to the individual. The tissues of the arm are of the same material and are affected in much the same way and by the same thing as tissues in the other parts of the body.

"By injecting the extract the tissues are stimulated to produce what we call antibodies to combat the poison. The principle is the same as in typhoid vaccination, except that the doses are much smaller."

One of the most interesting cases of the use of this test in connection with occupational diseases was that of a baker who in 1914 came to Dr. Turnbull, who was then at the Massachusetts General Hospital. The baker was suffering from chronic inflammation of the nose, eyes and throat which nothing seemed to help.

He was requested to bring samples of the flour used in the bakery to the hospital and from it a paste was made. This was used after the fashion of smallpox vaccine. Swelling and redness followed. The baker was ordered to stop eating wheat bread and to keep away from the bakery for a time. Injections with an extract of the wheat were given and his trouble was cured.

One patient, who had suffered from asthma for 20 years, was found to be free from it if she gave up eating lamb. After this was known, lamb was given and her asthma returned. She was able to eat the meat without further trouble. Other patients suffered from eczema, caused by eating fish, and were cured by giving up the fish or by treatment or both.

Figure 1 (Continued). See legend on opposite page

extracts (with too many having too little relevance to reality) was compounded by the use of screening mixtures of (even unrelated) test materials. Misinterpretations and erroneous conclusions were the result of attempted short cuts.¹⁶² The potential positive reactivity of an individual allergen could be lost by dilution; conversely a summation of positive reactive components might exceed the limits of safety and risk inducing a constitutional reaction.

Placing complete reliance on results of skin tests without relating the reacting allergen-reagent to clinical study unfortunately amounted to more than a capricious exercise or major cause of error. Serious, near

fatal, constitutional reactions were brought about by failure to appreciate the importance of clinical histories that should have dictated foregoing the skin test (done primarily for the academic value of verification); failure to use diluted extracts; and failure to jeopardize safety on first use of extracts, especially without having definitive information on the proper concentrations to be employed.¹⁶⁷

This confused state of affairs had come about as the result of a combination of several circumstances and factors. With the introduction of the subject of allergy and skin testing into the medical literature, the public flow of Walker's reports published between 1917 and 1920¹⁹¹ expeditiously popularized the "use of the scratch tests by internists and pediatricians, most of whom learned the techniques by reading his publications."¹⁶⁷ Following the establishments of Cooke's allergy clinic in 1918 and Coca's Laboratory of Applied Immunology in 1919, the procurement of allergen extracts readily was controlled. Extracts were made available only to those physicians who had taken Coca's course or who were affiliated with Cooke's clinic or the clinics he and his staff had been responsible for seeding.³⁹ This, however, soon changed with the entrance of commercialism.

In 1918, Roger Wodehouse, armed with a base of information and experience with Walker, joined the Arlington Chemical Company of Yonkers as a botanist. Subsequently, as Scientific Director he established its Hay Fever Laboratory, and Arlington-produced allergen extracts then became available for purchase on the open market. Concurrently, O. C. Durham's expertise in performing plant and pollen surveys for Swan-Myers Company in Indianapolis ultimately contributed to the increased production and offering of commercial pollen extracts when Abbot Laboratories took over the former firm. By 1929, the *Journal of Allergy*, the first English language journal in the new field, was published. Its initiating issue carried advertisements of six firms offering the sale of allergen extracts for skin testing: Parke Davis, Lederle, Mulford, Cutter, Swan-Myers, and Arlington.

For physicians not wishing or ready to invest in procuring materials and setting up their own skin testing resource, alternative services became available (F. Rackemann, R. Kern, M. Walzer, L. Tuft, H. Alexander, S. Feinberg, G. Piness, L. Crip, personal communications to the author [SCG], 1969). Appointments could be arranged for patients to be tested in a physician's office by a visiting technician—representative of, or with material obtained from, a commercial pharmaceutical source. Another setting for obtaining skin testing service evolved from the concept of the procedure as an appropriate function to come under the purview of hospital laboratories. Through this medium, clinical pathologists were introduced and attracted to and entered the private practice of allergy (e.g., Milton Cohen [Cole], Warren T. Vaughan, J. Harvey Black) (M. Cole, personal communication to the author [SGC], 1969).

For convenience and simplification of practical needs, thoughtful suggestions and recommendations were offered on the basis of the experience and rational trials. Vaughan's botanical classification of edi-

bles, which noted that antigenic relationships followed genetic relationships, systemized group testing.¹⁸⁰ Feinberg's modification of biologic classification of all types of allergens highlighted the most allergenically important member of each group as the primary representative agent for testing.⁶⁷ Thommen's postulates for selection of geographically defined pollens required five criteria of characterization for acceptance as source materials for preparation and use of skin test extracts. Incriminated pollens must: (1) have excitant quality, (2) effect pollination through wind borne carriage, (3) have buoyancy, (4) be disseminated widely and produced in large quantities, (5) and be derived from plants that have abundant and widespread distribution.¹⁶⁴

One important fact in the prelude to setting up the mechanism of certification in allergy is represented in the observation of a founding member: "In the 1930s more and more doctors were beginning to become interested in allergy. To develop a large practice was easy, for patients and their family doctors were intrigued by skin tests . . . we realized that something should be done to control the increasing number of skin testers who did not know too much about the treatment of asthma, and that gave rise to the idea of certification." (F. M. Rackemann, personal communication to the author [SGC], 1968.)

Moving in this direction in 1932, the SSAAC and ASA set up a Joint Committee on Survey and Standardization. In developing criteria for approval of allergy clinics, the Committee spelled out among its requirements "adequate materials for skin testing by scratch or intracutaneous methods, and preferably both; facilities for keeping intracutaneous solutions fresh, particularly after being diluted; and an adequate record system."³⁶ The Committee's list of recommended allergens for routine skin testing of clinic and private practice patients was comprised of common pollens and perennial inhalants (including three sources of feathers and ten danders), and 28 foods.

All this activity, concern, and orchestration in the United States were in marked contrast to the situation in Europe where allergy was not yet developing into a specialty. Enthusiastic reports by American pioneers on the value of skin testing as a diagnostic tool in the study of asthma offered too little reason for belief and support in Europe. At a symposium on asthma in Germany in 1926,⁹² internists in agreement criticized and condemned the procedure as a method of diagnosing hypersensitiveness. Little by little, the adaptation and use of Cooke's New York Hospital materials and techniques in a diagnostic clinic in Berlin¹¹¹ helped to overcome contention that Europeans were not sensitive to atopens that were prevalent in America. Also, erroneous belief that hypersensitiveness was not an important factor in the cause of asthma required time and experience to refute and dispel.²⁰⁰

In England in the 1940s and 1950s, allergy continued to be considered just another subject area relevant to varied clinical fields. From British colleagues we learned that patients with asthma and rhinitis for the most part came under the care of practitioners of general medicine and otolaryngologists. In the hands of physicians engaged in those areas,

the use of skin tests had a primary limited objective—solely to ascertain whether the affected subject was atopic. For such diagnostic inquiry the considered need could be met by skin tests with few allergen extracts (e.g., house dust, two species of animal hairs, and two to three representative tree and grass pollens and commonly incriminated foods) (author's [SGC] notes and recollections).

In contrast to the United States, where escalating interest and active undertakings in allergy was shaping its progressive development into a specialized field of practice, it would take many more years—up until the year 2000—before allergy became a recognized specialty of consultants in British medicine. Consequently the diagnostic aid of skin testing found a place in only a few established London clinics that paid attention to serving the needs of asthma and hay fever patients. Of the hospital clinics started by John Freeman (as an outgrowth of his introduction of immunization therapy of pollenosis), William Frankland told his experience (personal communication to the author [SGC], 2000):

"I can assure you that, when I went to St. Mary's Allergy Clinic in 1946, we used to do very extensive skin prick tests on patients . . . very lucky that we had our own laboratory to make up all our extracts and all our vaccine . . . you may hardly believe the number of patients (largely seasonal hay fever, some with associated pollen asthma) we could deal with in a short time, but I was criticized when the special clinic one Spring had tested over 6000 patients—I was told I had to reduce the numbers because they were overtaking the Hospital."

By 1948, a mix of physicians and medical scientists (40 in number) had formed the British Association of Allergists (later the British Society for Allergy and Clinical Immunology [BSACI]). Of Vera Walker, the first president of the society, and her work as an ophthalmologist, Frankland recalled, "In the 1950s, she and others did intradermal tests with bacteria, I believe, and she did carry out quite a lot of ordinary skin prick tests on patients" (W. Frankland, personal communication, 2000). As the first society secretary continuing into a 15-year tenure, Frankland, in a historical review, wrote of a session at the first (1948) meeting on "the use of skin testing in asthma and hay fever. The conclusion was that skin testing was an aid to scientific research, but it might be useless and indeed, misleading."⁷²

SKIN TESTS AND CLINICAL RELEVANCE

The initiating contributions of Goodale,⁷⁶ Schloss,¹⁴⁸ and Walker¹⁸⁹ on the scratch test during the period from 1908 to 1917 and Cooke's⁴⁰ development of the intracutaneous counterpart were pivotal in the advancement of studies on allergic diseases. Early work that concentrated on improving testing techniques and laboratory preparation of materials resulted in excessive emphasis on the importance of diagnostic proce-

dures. Pitfalls encountered along the way soon pointed to problems that would continue to be faced in placing undue dependence on the results of skin testing to expedite progress in the field. With careful observations, accumulating experiences, and critical correlations, early heralding of the whealing response as an all-or-nothing phenomenon gave way to finding a valid place for skin testing in the scheme of clinical and research medicine.

State of the Art

Ten years after Walker's initial reports, Duke reported:

"One's first impression of skin testing is likely to be one of disappointment. If he is so unfortunate as to be seriously misled by them . . . The fault lies in defects in the method itself. In order to interpret correctly, it is necessary to bear in mind two facts: (1) that a strong positive cutaneous reaction with a given substance does not by any means indicate that it is responsible for an illness, and (2) that a negative reaction with a given substance does not exclude it as a cause of illness."⁵⁵

Fifteen years after Cooke's clinical initiative, Walzer and Thommen reported:

"Skin testing, as a means of diagnosis, has many faults. A careful analysis and study of the various aspects of the subject, in the last 15 years, has exposed most of these limitations. The Prausnitz-Küstner phenomenon has given us a most helpful means of checking the values of each test. The ophthalmic, nasal, and constitutional tests offer further aid in confirming test values . . . To one who has had intimate experiences in skin testing for a long period of time, its value in a large percentage of cases is unquestioned . . . The severest criticism of the procedure had come from those who have had little or no experience with it, and expect too much from it. Disappointments unnumbered await the novice who is misguided enough to believe that the mere application of an extract into a skin scratch or its intracutaneous injection, comprises the entire diagnostic procedure . . . Those who have made a thorough study of the subject are satisfied that skin testing is at present the most valuable single diagnostic procedure available in hay fever and asthma. Although far from perfect as a technic . . . it [is] of inestimable worth. Until a better substitution is found, it may be considered indispensable."⁵⁶

Another 15 years later, Walzer stated:

"Today, while the importance of the skin test is fully appreciated, its limitations as a diagnostic procedure are also realized. The clinician has learned that the skin test is, at best, only a relative crude diagnostic technic which, though relatively simple in its performance, is subject to many pitfalls. Considerable experience is needed for its proper application and for the effective

interpretation of the reactions obtained with it. It is now common knowledge that the skin test is of diagnostic aid only in certain forms of allergic illnesses. In others, even when skillfully performed, it is either of limited value or completely useless."¹⁹⁹

Positive and Negative Skin Tests: True or False

Even in the hands of knowledgeable physician pioneers of the emerging field of allergy, anticipation of the maximum skin-test-positive result in the sensitized patient often failed to materialize. With accumulating experience, there were increasing numbers of reports of both the inability to confirm demonstrable allergen-symptom relationships by whealing responses and expressions of positive whealing reactions in the absence of expressions of hypersensitivity disorders. Variable and inconsistent results were found on duplicate testing with extracts of identical antigens prepared in different laboratories, even with the same extract repeated on different days.

Proffered explanations dealt with various individual or combinations of factors, components, and steps in the testing process. Regarding technique, considerations included the depth of scratch or needle insertion and amount of solution injected intracutaneously,¹³⁹ nonspecific whealing secondary to tissue trauma, particularly on hyperirritable (dermatographic) skin, and pseudopositive tests induced by the influence of strong reactions on tests placed at insufficiently spaced adjacent sites.²⁰⁵ Several reasons were offered for the inadequacy of testing materials. Those concerned with improper preparation^{35, 208, 209} included: the use of reagents with ineffective or deficient chemical properties to attain aqueous extraction of lipid-freed protein atopens; failure to use adequate chemical buffering to maintain the requisite pH and bactericidal and stabilizing agents (toluol and glycerin) which resulted in the deterioration of protein extracts; unnecessary cleansing of animal hair sources that resulted in the loss of the desired epithelial danders and cat saliva responsible for their allergenic properties; excessively vigorous attempts to achieve extracts of purified protein resulting in loss of allergenicity; and inability to remove nonspecific irritant properties inherent in some extraneous components.

Ultimately, through experience augmented by careful observations, sorting out, and critical evaluations, salient points evolved.¹⁹⁹ Cutaneous reactions could result from more than a single immune mechanism, explaining variability of results. In atopic disorders, immediate reactions reflected only the presence of reaginic (skin-sensitizing) antibodies, and were not definitively diagnostic. Beyond skin-test-detectable reagents that reacted with corresponding allergens, expressions of disease required responsive, targeted shock tissue. Positive skin tests were not uniquely pathognomonic of atopic hypersensitiveness—nonatopic humans produced reagins to *Ascaris* parasitic helminths, horse serum, insulin, and liver extract. Identification of uniform attachment of reaginic antibodies

to cutaneous and mucous membrane tissues validated leads derived from skin testing in patients with symptoms limited to respiratory, ocular, or gastrointestinal involvement. In certain atopic-simulating disorders (chronic urticaria and angiodema, papular urticaria, vernal catarrh, drug reactions) skin testing was recognized to be of little or no practical value.

Perceived lack of relevance of skin test results to clinical disorders was particularly evident in two instances: (1) the high prevalence of skin test reactions (as great as 80%) in patients representing a wide spectrum of symptoms (many neither respiratory nor perennial) and even in asymptomatic subjects²⁰⁰; and (2) in contrast there were patients who exhibited atopic manifestations, especially marked degrees of acute urticaria, angioedema, rhinitis, and severe acute paroxysmal asthma unmistakably related to aspirin, yet they demonstrated negative skin test reactions.

In the case of house dust extract, a prevailing opinion attributed the ubiquitous reactivity to the multiplicity of allergens derived from household collections of furnishings, contents, products of deterioration, linters, microbial contaminants, and contributions from materials used by occupants. The extracts were thus presumed to cover most, if not all, perennial environmental inhalants to which one could be allergic.⁴ The concept, however, was not offset by the failure to bypass questionable, if not false-positive, test reactions with extracts of autogenous house dust (prepared from and limited to collections made in the patient's own home)⁸⁹ or with a "purified extract" (Endodust)¹⁷ aimed at eliminating the irritating, histamine-releasing endotoxin components for nonspecific whealing.¹²⁰ Ultimately, there was the breakthrough identification of mite species as the source of house dust reactions.¹⁸³ Consequently, the inability to demonstrate the existence of a single characteristic entity in stock house dust extract and its use in skin testing were relegated to academic issues.

For test demonstration of aspirin sensitivity (as in the case of most drugs in common use that had been implicated in adverse reactions: quinine, barbiturates, phenolphthalein, arsphenamine, bromides, iodides) it was not possible to elicit positive skin test reactions. The serious nature of aspirin (asa)-induced asthmatic attacks and the severity of the Samter's (aspirin triad) syndrome (asthma, nasal polyps, and aspirin sensitivity) gave special reason and incentive to establish a diagnostic aid. Conventional scratch (with moistened powdered asa) and intracutaneous (with weakly soluble asa in saline) tests were nonproductive and posed a sufficiently valid danger to be contraindicated. Absorption of the smallest amount of the compound from the test site into the blood stream could pose a potentially fatal risk. Qualified acceptance of the French method (daily application to or into the skin by scratch or cutaneous puncture of gradually increasing amount) could be considered.²⁰⁰ Substitution of other salicylate compounds (e.g., sodium salicylate) and extracts of foods with natural salicylate content were equally negative; the adverse determinant seemed to reside in the acetyl radical.

In differentiation from asa's nonreactive feature, the sensitizing character of other drugs that similarly elicited negative responses to conventional skin testing (antipyrine, phenolphthalein, potassium iodide) could be demonstrated by specially designed tests. In fixed drug eruptions, manifestations of hypersensitiveness could be passively transferred by autotransplantation of the drug-induced lesion and through the medium of fluid taken from blisters raised at involved sites (cell transfer equivalent of the P-K serum transfer phenomenon [i.e., Urbach-Koenigstein technique].^{171, 172} Additionally, although epidermal and cutaneous tests with these drugs also failed to elicit reactions on normal skin, unlike asa, they produced positive responses when tested on previously affected localized sites.¹⁷¹

Other approaches included the use of aspirin conjugates²¹¹ and blood recovered from a normal subject after taking asa tablets; the latter with the thought that a possibly active, physiologically degraded asa product might make the serum a suitable test reagent (P. Blank, personal communication to the author [SGC], 1948). Against the background of failed skin tests, Duke proposed a systemic test that involved placing a small granule on the tongue, watching for the development of symptoms, and then rinsing the mouth with a weak acid (vinegar in water) to terminate the reaction.⁵⁶ Too risky for routine use, the procedure could be advocated only when a patient's history was indefinite or when clinical information indicated a critical need for aspirin therapy.

One might question whether the negative skin test property of an asthma-causing drug should have cautioned Cooke against casting his 1932 tetentina definitive tone. "In all types asthma is fundamentally the expression of an allergic reaction."⁴⁸ Premature by 44 years, biomedical research in 1976 had uncovered the pathogenesis of skin test negative-aspirin asthma as not atopic but inflammatory, as a consequence of inhibition of the cyclooxygenase mediated pathway.¹⁷⁵

The converse, positive whealing skin test reactions to drugs in the absence of any expressions of clinical manifestations similarly led to a rewarding investigational search.¹⁵⁵ The production of immediate local urticarial lesions in response to introduction of certain trial chemicals apart from any atopic relevance or immediate hypersensitivity mechanism, by both scratch test or puncture-prick techniques, duplicated the circumstances responsible for histamine- and allergen-induced wheals. The positive reacting agents were primarily morphine and its esters, atropine, calcium chloride, urea, and formaldehyde. The experimental conditions pertinent to local action that ruled out systemic effects and the lack of relevance to capillary paralysis, acidosis, inflammatory or sensory irritation, or osmosis set the stage for evolving information on and identification of nonspecific histamine-releasing agents.

The finding that large cross-linked polysaccharide molecules could have antigenic-allergenic properties identical to proteins was an established immune phenomenon.⁸³ Demonstrations that high-molecular-weight dextran solution evoked immediate positive whealing reactions on initial skin tests thus logically could be attributed to sensitizations to

dextran components of colonizing bacteria (e.g., *Leuconostoc mesenteroides*, streptococci) in the respiratory or intestinal tracts or from capsular polysaccharides of infecting pneumococci. The demonstration of skin test responses in the absence of corresponding antibodies was not an unusual finding, however.⁹¹ Through skin test identification of such reactive properties, dextrans were added to the list of nonspecific histamine-releasing agents²⁰⁴ initially associated with the effects of histamine and codeine.¹⁵⁴

Etiologic factors were sought through skin-test-based inquiries to explain negative cutaneous reactions to milk related to clinical evidence for a role in effecting characteristic symptoms. The indirect transfer of an atopen contained in an otherwise nonirritating dietary source was first suspected from anecdotal circumstantial associations of peanut, ragweed, and flaxseed allergies to the consumption of milk from cows fed peanut hay, ragweed tops, and flaxseed meal.¹⁸² The use of skin tests in another case report of a 6-month-old child fed only modified cow's milk associated her infantile atopic dermatitis with a positive reaction to wheat but a negative reaction to milk. Her symptoms resolved when the diet was changed from milk obtained from cows fed on bran to milk from cows fed on green wheat pastures. This observation led to the impression that cows feeding on wheat bran may have served as the source of the offending allergen.¹¹

Validation of this concept was achieved through the imaginative use of the skin test technique. In 1922, Herrmann applied ragweed extracts and an extract of milk prepared from cows fed ragweed tops to scratch test sites on the arms of two subjects—one who had hay fever and another who did not.⁸⁵ Positive reactions to both extracts were noted in the sensitive individual but not in the normal control. In definitive testing, when both subjects drank ragweed-containing milk, the control subject had no untoward effects; the ragweed-sensitive patient who had been free of hay fever symptoms during the prior 24 hours experienced ocular burning, lacrimation, and itching of the roof of mouth within 30 minutes of ingestion and within 1 hour suffered a hay fever attack followed by asthmatic symptoms later that evening. Testing of a second hay fever patient displayed similar reactions and corroborated the belief that ingestion of ragweed-containing milk could serve as the medium for transmission of an active pollen allergen.

Eight years later, Donnelly, intrigued by early reports of infants fed exclusively breast milk who were afflicted with eczema or urticaria, investigated whether egg protein eaten by a lactating woman could be eliminated in her milk in detectable quantities.⁵⁴ Concentrated whey of breast milk obtained from three of eight women after each ate only raw eggs on an empty stomach was tested at passive-transfer sites in two of four normal recipients; whey gave a greater specific reaction to egg white than to the control extract. Additionally, when skin test reactions obtained with the breast-milk whey were compared with those elicited from known dilutions of egg white, appreciable concentrations of egg white were found in the whey sample.⁵⁴ Once again, through inventive

modified use of direct skin test and passive transfer techniques, an indirect food source was validated as a unique mode of antigen delivery, and the causative agent dispersed in an otherwise benign compound also could be identified and measured.

SKIN TESTS AS INVESTIGATIONAL TOOLS

With the clearing of confusion that had been created by misinterpretations and misunderstandings of the nature of false-positive and false-negative reactions, the modality of skin testing was introduced into hypersensitivity research. The original intent of providing physicians with a diagnostic aid for detection or verification of suspected allergens as etiologic agents found new adaptations in clinical practice. The time of development of specifically designed reagents and sophisticated technology to initiate laboratory studies of "hypersensitiveness and clinical anaphylaxis" had not yet arrived. Imaginative usage of routine scratch and intracutaneous methods and insightful interpretations and applications of test results contributed to expansion of the practical knowledge base of an evolving medical specialty. Selected samples of new information thus uncovered through the pioneering use of allergen skin tests are summarized below.

Differentiation of individual types of skin test reactors led to definitions of pathogenetic features of disorders with overlapping characteristic symptoms. In 1918, there was Rackemann's delineation of allergen-related and intrinsic asthma¹³¹; 10 years later, there was Vallery-Radot's demonstration of nonallergic, paroxysmal (vasomotor) rhinitis¹⁷³; and in 1932, critical characteristics of allergen-related atopic dermatitis were identified within the morphologically similar neurodermatitis group.¹⁶⁰

In 1934, Tuft's differentiation of horse serum (and shared horse dander) allergy and serum sickness established skin and ophthalmic test criteria critical for the prevention of anaphylactic shock in the preantibiotic era of passive immunization with tetanus and diphtheria antitoxins. These guidelines stood in contrast to the nonpredictive reliability of a negative skin test foreshadowing the developments of a serum-sickness-pattern adverse reaction.¹⁶⁹ Also, in instances of hyperactive immunization with tetanus toxoid, delayed test reactions reflected Arthus (immune complex) reactions caused by pre-existing high levels of circulating antitoxin rather than indication of reagin-mediated sensitization.

With the discovery of skin-sensitizing serum reagins, the concept of desensitization (later hyposensitization) replaced Noon and Freeman's belief that injections of pollen extracts induced antitoxic immunity.^{73, 114} Visualization of a hyposensitizing mechanism also became untenable with the finding that clinical improvement occurred without demonstrable neutralization of reagin antibody. Study of effects on passive transfer (P-K) reactions demonstrated an allergen-specific inhibiting factor in sera of treated patients.⁴³ Blocking antibody was thus identified through the investigative medium of skin tests.

Elucidation of the reagin-inducing contributions of beef and pork source derivatives and the insulin molecule itself enabled the selection of specific allergen-free preparations for insulin replacement therapy of sensitized diabetic patients.¹¹² Additionally relevant was the demonstration of independence of identity and action of antibodies responsible for insulin allergy and immune-mediated insulin resistance.¹⁰¹

The identification of allergy to cow's milk resided in one specific fraction: lactoglobulin, the most frequent skin test reactor. Its destruction by heat and the finding of negative test reactions to the least common offending fractions (lactoglobulin and casein) provided a guideline for dietary elimination.⁵¹

The biologic phenomena of variable genetic diversity and similarity were reflected in differences of skin test reactivity to molecular fractions of lactalbumin and lactoglobulin which demonstrated species-specific individualities. In contrast, milk casein and egg white albumins of hens, ducks, and a spectrum of bird species shared identity.²⁰³

Early workers in the field were well aware that the preparation of allergenic extracts required fresh, raw foodstuffs as source material.¹²¹ Easily demonstrated was the absence of skin test reactivity paralleling natural deteriorating processes in fruits and vegetables, the effect of heat on egg and milk, and experiences of bakers who manifested asthma while working with wheat and rye flour but could eat baked bread without similar adverse effects. Understanding the strange nature of the reverse phenomenon, creation of allergens by protein denaturation, was aided by skin test leads. In the seminal experiment on the passive transfer (P-K) reaction that provided earliest insights on reagin antibodies, Kustner reacted only to the cooked, but not the raw, fish extract.¹³⁴ Other relevant instances were Duke's citation of Meixner's patient whose asthmatic sensitivity to peanut was expressed only after roasting and salting the peanuts⁵⁵ and Rowe's observation on hypersensitiveness to cooked but not raw fruits.¹⁴³ From only the brief anecdotal references provided, however, it was not clear whether the patients' manifestations were evaluated by skin test reactivity.

Further use of the skin test facilitated the investigation of epidemiologic questions. Occurrences of endemic asthma within a confined geographic location were traced to exposure to a common, environmentally disseminated atopen. The finding also served to challenge the underlying epidemiologic principle that normal individuals could develop asthma solely through exposure. In 1928, the fact that 30 individuals living within a 1-mile radius of a castor oil mill developed asthma was considered unusual in that the prevalence of castor-bean sensitivity was rare.⁶⁹ In all the affected patients, scratch tests to castor-bean extract demonstrated immediate whealing reactions; of these patients, half had a personal or familial history of atopic hypersensitiveness. Pursuing the lead provided by positive skin test implications, it was found that the responsible atopen derived from the dust of ground and extracted castor beans was emitted into external air through mill exhaust pipes. Resultant asthmatic attacks were thus dependent on the direction of the wind.

Other persons who lived near the mill and had suffered asthma caused by a variety of allergenic agents (but not caused by castor bean exposure) supported the contributory role of specific hypersensitivity rather than locality.

Thirty-six years later, investigation was undertaken to follow a circumstantial lead that suspected cockroaches to be source allergens in indigent asthmatic patients throughout many US metropolitan cities. In 1964 Bernton and Brown studied 253 normal individuals and 114 patients classified as allergic to determine the incidence of skin sensitivity to this prevalent, pesky insect.¹⁴ Of the 303 adults and 64 children tested, positive skin test results were significantly higher in the allergic group compared with the control group. The finding of skin-sensitizing antibodies in blood sera in the positive reactors gave added significance to the skin test results. The cockroach antigen also was found in food contamination. Household surveys indicated that antigen exposure came from direct contact with cockroaches, body parts, products, and food contamination. In light of these findings and the fact that cockroach infestation was so widespread, the recommendation to routinely include this allergen in skin tests prevailed.

Similarly, during this same period, the greater-than-usual incidence of allergy in children in Norway suggested an endemic prevalence. The circumstance of residence and exposures in a localized area where commercial fishing provided the primary occupational pursuit and the availability of fish as a frequent food led to the use of skin tests for diagnostic leads.¹ In 89 children, fish was identified by clinical observations as a cause of asthma and urticaria; all demonstrated positive skin test reactions to fish extract. Although the prevalence of hypersensitivity to fish was higher than usual in this circumstance, it was found to be a singular cause of asthma in only 5%. Conversely, more than three quarters of these patients manifested asthma from other allergens, including other foods, house dust, animal danders, pollen, molds, and feathers. Accordingly, the pre-existing belief that exposure to fish alone induced endemic asthma in children was nullified.

In each of the three epidemiologic studies, the initiatives to investigate suspected allergens were aided by the use of skin tests. Findings through this technique implicated direct relationships between specific allergens and expressions of asthma. The results could not substantiate an existing belief that asthma occurred as an endemic disease in the populations studied. Additionally, the outcomes of these evaluations were pertinent in providing reason to refute the inclusion of "endemic asthma" in proposed classifications of this disorder.

Early thought held that in the evolutionary scale of immune phenomena, experimentally induced anaphylaxis—as demonstrated in dog, guinea pig, and rabbit models—represented the limit of sensitization development and expression in lower animals. An important initiating step in ultimately dispelling this belief were the initially reported demonstrations of spontaneous manifestations in affected animals that suggested similarities to atopic human disorders. Of particular interest as

subjects for inquiry were hay fever in cattle²⁰¹ and dogs²⁰⁷ and eczema and urticaria in dogs¹²⁷ and cats.¹⁰⁷ Information was then sought to provide insights, if not elucidation, of possible etiologic mechanisms such as pathophysiologic or immune system functional deviations, particularly if atopy could be shown not to be a restricted human marker. Evolution and genetic-oriented biologists were intrigued by opportunities to study whether removal from wild natural habitats to substituted new environmental exposures and foods influenced generational development of naturally occurring allergic disorders only in domesticated species.

In demonstrations of immediate intracutaneous responses to food extracts, eczema and erythematous urticarial eruptions were found to be the primary manifestations of allergy in dogs.^{107, 127} Their cutaneous expressions equated with human manifestations of allergic rhinitis, asthma and atopic dermatitis. Respiratory tract involvements were seen in less frequent instances of seasonal pollen rhinitis; nasal and ocular symptoms were invoked, on direct mucosal testing and positive skin test reactions to weed pollen extracts.²⁰⁷ In many instances, clinical observations validated the relevance of skin test reactions to symptoms. In cows who presumably had been exposed to ragweed in their pasturage, positive skin test reactions and serum-transmissible reactions to reagins were demonstrated.

In relating intestinal helminth parasites to urticaria in dogs, the demonstration of passively transferable reaginic serum gave added credence to leads furnished by direct skin tests to ascaris antigen.²⁴ This finding was consistent with that of human experience—zoologists whose episodes of urticaria, pruritus, rhinitis, and ocular inflammation were associated with handling ascaris worms or products in their laboratory settings.⁷⁵

In the study of heaves, an asthma-like syndrome in horses, Lowell demonstrated positive skin test reactions to hay extract. Observations of attacks associated with feeding of hay and of clearing of cough and quieting of respiratory difficulty after removal of hay from the diet confirmed the interpretation of skin test results.¹⁰²

In contrast to the delayed skin test responses characteristic of Arthus reactions evoked in guinea pigs subjected to experimentally induced anaphylactic sensitization, those reactions associated with naturally occurring allergic disorders in cattle, dogs, and cats were of the immediate hypersensitivity type (somewhat slower in time of development in horses). Nevertheless, experimental immunizations and development of new testing techniques (active and passive cutaneous sensitization) in guinea pigs and rats provided laboratory models for study of skin-sensitizing antibodies.¹²⁷

Through these studies, investigations using skin test materials and methods provided meaningful new knowledge and informational leads, findings, and interpretations that were directly applicable to materials, methods, and treatment practices in veterinary medicine and development of advanced experimental models for allergic disease research.

BACTERIAL ALLERGY

Genesis of Concept

The concept of "bacterial allergy"—belief that development of hypersensitiveness to bacterial molecular components, fractions, and their products could engender atopic disorders—grew out of a blending of experimental findings and clinical observations. A starting point was provided in 1891 by Koch's seminal demonstration of altered immune tissue reactivity in the tuberculous-infected guinea pig.⁹³ On another and unrelated track, Meltzer in 1910 gave a new direction to understanding asthma in viewing its pathogenesis and symptomatic expression as a counterpart of the bronchial smooth-muscle constriction of guinea pig anaphylaxis.¹⁰⁶ A bridge between the human disorder and the animal model took shape in Rosenau and Anderson's extended study of anaphylactic shock reporting that the phenomenon they had described as a function of sensitization to foreign (horse) serum¹⁴¹ could be reproduced with microbial-derived antigens (extracts of yeast and colon, hay, anthrax, tubercle, and typhoid bacilli).¹⁴²

Physicians pioneering the development of the field of allergy found circumstantial reason in their accumulating experiences and in case reports for ascribing relevance to perceived inter-relationships of infection, sensitization and asthma. (1) Episodes of paroxysmal asthma frequently were triggered by acute upper respiratory tract infections. (2) The onset of chronic asthma, rhinitis, and urticaria often were preceded by a respiratory infectious disease (e.g., pneumonia, influenza, bronchitis; in children, pertussis and measles). (3) Chronic hyperplastic sinusitis and nasal polyps with bacterial colonization, mucopurulent secretion, and eosinophilic cytology associated with persistent asthma constituted a familiar syndrome. (4) Longstanding allergic manifestations and asthma occasionally were relieved promptly by drainage or eradication of a focus of infection. (5) Exacerbation or reproduction of allergic and asthmatic symptoms frequently followed injection of an autogenous, or sometimes even a stock, vaccine.

The theoretic process implicating bacterial etiologic factors in this clinical spectrum (otherwise difficult, if not impossible to prove) found additional interpretive support in the pivotal contribution of Rackemann's classification of asthma.¹³¹ In the absence of a definitive explanation traceable to skin test-positive, extrinsic, inhalant, or food allergens, his crystallization of nonatopic intrinsic asthma placed hypersensitivity to microbial-derived agents high among possible causal mechanisms.

Analytic Role of Skin Tests

As with intrinsic asthma (ultimately to be recognized and appreciated semantically under the definition of idiopathic asthma), bacterial allergy remained a diagnosis by exclusion for the want of other than

circumstantial associations. For evaluation of patients who presented with atopic-like disorders yet failed to react to allergenic inhalants and ingestants, early workers introduced diagnostic materials to test assumptions of the existence of bacterial sensitivity as an *in vivo* functional entity. In searches for investigational leads or confirmatory aids, finding lack of uniformity and differences in interpretive criteria of skin tests with microbial derivatives confounded clarification of the issue. In the outcome of controversial and biased pro and con reports, a place for skin tests in the diagnosis of bacterial allergy and identification of specific agents remained unresolved. The question of corresponding therapeutic application by (hyposensitizing) injections of bacterial vaccines was equally unanswered.

The idea of using bacterial proteins in skin tests appeared in a 1916 report on vasomotor rhinitis by Goodale.⁷⁸ The following year saw the first of Walker's publications on scratch test reactions obtained with dried protein powders prepared from staphylococcal, streptococcal, and micrococcal strains isolated from nasal secretions and sputum samples of his asthmatic patients.¹⁹⁰ His extensive series that followed,¹⁹¹ which described immediate and delayed reactions that equated with the significance given to his findings with inhalant extracts, did much to popularize the subject.

In retrospect, the variability of Walker's test responses, whether immediate or delayed, could be found in experimental animal studies 7 years earlier. Using Koch's tuberculous guinea pig model, Baldwin demonstrated anaphylactic sensitization with extracts of ground tubercle bacilli extracts, but only active infection could induce tuberculin cutaneous reactions.¹⁰ In 1921, Zinsser's work explained the dichotomy. Immediate wheal-flare skin reactivity lay in the limited sensitizing action of the bacterial nucleoprotein fraction, whereas the delayed tuberculin type reaction—demonstrable only as a product of active infection—was attributed to a nonprotein "residue antigen."²¹³ Of special interest was Zinsser's subsequent identification of nonprotein (carbohydrate) substances that were otherwise chemically similar to that of the tubercle bacillus in a variety of other bacteria.²¹⁴ This fact may be relevant to explaining the delayed cutaneous reactions in Walker's and other workers' related skin test findings.

At this point, Cooke's postulates assumed pertinence in satisfying the etiologic importance for implicating any substance suspected to play a causative role in a case of hypersensitiveness. According to Cooke, demonstration of sensitization required a positive local cutaneous or ophthalmic reaction, reproduction of the allergic manifestation at will by administration of or on exposure to the suspected provoking agent, and evidence of contact between affected individual and the specific substance in question.⁴⁶

Accordingly, Cooke found reason for criticism in noting that Walker did not show proof that positive reactions to bacterial proteins had any bearing on the asthmatic condition or were etiologically diagnostic in any of the case reports and further had shown only favorable responses

to vaccine treatment irrespective of reactions to bacterial proteins (thus a nonspecific effect). In Cooke's series of studies using intracutaneous technique (compared with Walker's scratch method with identically prepared bacterial proteins), only two patients manifested positive reactions, which were to organisms of species that could not be isolated from respiratory tract secretions or induce constitutional reactions or injections.⁴⁷

During that concurrent period of the early 1920s, a number of other responsible clinical investigators, whose findings also were too inconsistent to agree with Walker, questioned the theoretical basis and value of his method and materials. Ultimately, 10 years after launching his series of studies, Walker conceded with the comment that "cutaneous [scratch] tests with bacterial proteins [did] not seem to be worth doing."¹⁸⁸ His credence in concept and objective nevertheless continued in noting merit in an alternative technical approach using killed bacteria in intracutaneous tests.¹⁸⁸

Walker's reference was to a method that Rackemann had introduced in 1920, in the belief that both the elicited immediate and delayed reactions were specific.¹³² Three years later—impressed by the finding that some vaccines produced "large local reactions in each and every individual"—Rackemann changed procedures but not materials. Subcutaneous injections replaced intracutaneous tests, and the amount of local reaction produced served as the determinant for matching each patient with the best-suited treatment vaccine.¹³³

Doubts cast on the validity of skin testing for 'bacterial allergy' were countered by reports of efficacy of injection treatment for asthma with respiratory bacterial vaccines. The practice continued for those clinicians and investigators who believed beneficial results could be attributed to hyposensitization (comparable with treatment of hay fever with pollen extracts) rather than to nonspecific effects. A popular modification introduced in 1924 by Thomas, Famulener, and Touart¹⁶³ was based on the empiric use of autogenous, rather than stock, respiratory bacterial vaccines. No differentiation was made in their acceptance of immediate and delayed reactions; the former was considered a reflection of anaphylactic sensitization, and the latter considered a presumed manifestation of "susceptibility to inflammatory effects of the [source] germ." Theoretical considerations were not considered or offered; both were believed to be of equal importance as indicators for selective therapeutic use of the reaction-inducing vaccine.

One of the major points against acceptance of or belief in the concept of bacterial allergy as either a primary etiologic or an associated triggering factor in atopic-like disorders was the predominance of delayed reactions evoked by intracutaneous testing with bacterial derivatives or products. Early whealing test responses were observed only infrequently. Even more exceptional were demonstrations of the immediate positive reactions by the passive transfer (PK) technique. The predominating conviction was that little if any objective basis could be found for comparison of asthma or rhinitis demonstrably associated with rea-

gin-mediated immediate reactions to pollen, inhalant, or food extracts with similar symptomatic disorders characterized by Arthus or tuberculin-type test reactions.

Proponents of intracutaneous tests with bacterial derived materials, based on belief in the reality of bacterial allergy and merit in its diagnostic and therapeutic applications, could be of two minds¹⁸¹: (1) the early whealing response in bacterial allergy, however infrequent, as in atopic allergy was more specific and predictive of a better therapeutic result than delayed reactions. Thus, efforts should be directed to develop vaccines capable of producing immediate type responses. (2) At the same time, delayed reactions were also specific; thus, there was validity in their interpretations and practical applications.

Reasons for taking this dichotomous position (beyond observations alone) perhaps could be found in certain aspects of immune phenomenon. In the study of infectious diseases, particularly when toxins or antigens derived from identified pathogenic bacteria and corresponding antibody responses had been characterized, the development of both allergy and immunity had been demonstrated. Immediate and/or delayed skin test reactions equally reflected the inter-relationships, interdependence,¹⁶⁵ and/or independence¹³⁸ of the responsible allergic and immune mechanisms. Clinically, in many infectious diseases the allergic state persisted after subsidence of the active disease process. Experimentally, repetitious intracutaneous injections of a foreign protein effected a chain of skin test reactions. Sequentially, a delayed inflammatory reaction developed first and then was followed by replacement with an immediate whealing reaction. On discontinuation of the injections, a stepwise loss of the induced reactions ensued in reverse order: the immediate first and the delayed the second to disappear.¹⁵² Additionally pertinent and applicable might have been Vaughn's conjecture based on observation that positive whealing reactivity could be suppressed or even replaced by delayed responses through persistent contact with the offending antigen.¹⁷⁹ A comparable circumstance could be envisioned in a chronically infected tissue site.

Consideration also was given to the possibility that fault for the failure to elicit definitive skin test reactions lay in the immunochemical state of testing antigens. According to one interpretation, the delayed reaction to intracutaneous bacterial testing was largely caused by the complexity of composition, physical state, and relatively low immunogenic properties of the antigens.²¹ Particularly pertinent could have been a lack of optimum correspondence on identity with antibody that had been produced to the original sensitizing antigen. In the Koch guinea pig model, it was shown that heat-killed tubercle bacilli induced the delayed-type skin test reaction, whereas same strain, ground, live tubercle bacilli elicited immediate wheal-erythema responses.²⁰²

In the case of pneumococci, antigen obtained by simple autolysis also effected immediate urticarial reactions.²¹⁵ Similarly, the problem might have originated in the manner in which the denatured antigens were prepared. Bacteria removed from growth, environment, and func-

tion in inflammatory-involved respiratory tract tissue, cultured on artificial media, and killed by heat or chemicals preliminary to preparations as a vaccine underwent intrinsic changes. As testing substances they could have differed markedly from their earlier states in original *in vivo* sources. Whether using an advanced sterilization technique or destroying the structural integrity of whole bacteria by grinding in a ball mill might have preserved sufficient antigenic identity remained a matter for conjecture.

Although the usefulness of skin testing for putative bacterial allergy remained unproven and controversial, equally empiric immunotherapy with mixed autogenous and stock respiratory bacterial, streptococcus, and staphylococcus vaccines and staphylococcal toxoid continued well into the 1960s. With the advent of anti-inflammatory corticosteroids and efficacious pharmacologic agents for the treatment of asthma and allergic rhinitis, belief in the concept of bacterial allergy and in the materials and methods for its testing remained an academic issue.

CONTACT ALLERGENS: THE PATCH TEST

Observations on the association of lip, tongue, and throat swellings and eating certain foods documented by Wilan in 1796²⁰⁶ awaited the 1912 pioneering studies by Schloss on food allergens for interpretation.¹⁴⁸ Schloss' demonstration of scratch test-induced whealing reactions to foods brought to light the role of immediate hypersensitivity in the symptom manifestations later to be known as the oral allergy syndrome¹²¹ and contact urticaria.¹⁵⁸ Neither application of this uncovered immune mechanism nor its diagnostic cutaneous test aids explained another earlier recognized type of plant contact-induced skin lesions.

Poison ivy dermatitis, then prevalent among North American Indians,¹⁵⁹ was known to the Massachusetts colonists²⁷ likely as early as the 1600s.²⁷ Although in 1819 the medical literature carried the first American case report of the eruption caused by contact with *Rhus toxicodendron*,²⁰⁵ the topical vesicant properties of the weed had been used at Scotland's Royal Infirmary in Edinburgh 25 years earlier for the treatment of paralysis.⁶

In 1839, reports appeared of inflammation of the hands from the handling of "flowers of grass" indigenous to Scotland.⁶⁴ With a subsequent series of journal articles beginning in 1890,²⁹ it became increasingly apparent that Europe had its own unique, continental, botanical annoyance—the sensitizing flowering plant, Primrose (*Primula obconica*, a species mentioned as early as c. 1101 in the '*Regimen Sanitatus Salerni*' among plants used to cure paralysis).

In each of these circumstances, the recognition that the skin of only some people could be affected provided leads for investigational studies. In England, Low (serving as his own subject) conducted skin tests with the juice from crushed *Primula* leaves¹⁰⁰ and, in the United States, Straus used poison ivy.¹⁵⁷ Both demonstrated that absence of responses to initial

applications in nonsusceptible individuals was followed by reactions indicative of generalized skin sensitization after incubation periods of 4 to 20 (usually 6 to 12) days. Test reactions to these contactant allergens in the presusceptible resembled those of delayed hypersensitivity only in their respective 48-hour response times. Unlike the antigen-antibody Arthus type of intracutaneous lesions, those lesions of epidermal sensitization (dependent on degree of sensitivity) varied from erythema to papule to vesicle formation. Additionally, unlike the test reagents for immediate and delayed hypersensitivities, the chemical character of those agents responsible for eczematous contact dermatitis was nonprotein. The (at least partial) identity of their respective oleoresin fractions accounted for cross reactivity among members of the *Primula* group and within the *Rhus* species, for example, ivy, oak, sumac, and later identified in related cashew nut shell, mango, ginkgo tree (Oriental lacquer), and Indian marking nut.

In 1783, preceding the first American case report of poison ivy dermatitis by some 40 years, Pearson (from Manchester, England) spoke of *eczema mercuriale*. In his surgical lectures, he described the idiosyncrasy of the diffuse skin eruptions appearing 8 to 10 days after beginning a course of mercury ointment inunction which was then used in the treatment of syphilis and gonorrhea.¹¹⁸ A precursor of (patch) testing by contact can be read into his notation of the confined reaction to "touching any part of the human body with mercurial ointment . . . [and] the accidental falling of a few grains of the *Hydragryrus Ruber* upon the skin."

With contact dermatitis ('*dermatitis venenata*') still to be appreciated as an immune-mediated allergic phenomenon, the scope of agents of cutaneously expressed "poisoning" expanded with case history information contributed as early as 1878.¹⁶ In addition to those agents of plant derivation, new contactant sources responsible for inducing eczematization through unbroken skin were iodoform surgical dressings,⁵⁰ arsenical pigment in colored wallpaper,²¹⁴ and chromate-dyed cloth.⁸¹ Together with mercurials (in contrast to pollen, inhalant, and food protein atops) their commonality was in the chemical character of molecular weight.

The landmark in recognizing the difference in skin eruptions following administration of therapeutic agents from those eruptions induced by application of drugs (chemicals) applied to skin came in 1896 with Josef Jadassohn's seminal work from Breslau, Germany. His observation involved a patient whose healed localized dermatitis caused by previous calomel (mercurous chloride) applications predisposed him to development of a generalized eruption and fever.⁸⁶ The complication that was triggered by taking a mercurial pharmaceutical (*hydragyrum oxydulum tannicum*) led to Jadassohn's design and introduction of the patch test.⁸⁶ Beyond providing a diagnostic aid in allergic eczematous dermatitis, the test initiated an understanding of contact-delayed hypersensitivity and the differentiation of its immune pathogenesis from atopic sensitization; also the identification of certain pharmaceutical agents

whose adverse effects could reach predisposed sensitized epidermis through dual routes, direct contact, and circulation after oral intake.

In extending Jadassohn's work studying iodoform as the sensitizing agent, Bruno Bloch further developed the patch test technique and value. Through its inventive use, he demonstrated that chemical contact sensitization, neither inducible in (or affecting) mucous membrane surfaces nor a humoral condition, was not a true anaphylactic variant. Bloch further provided interpretation of its cellular mediation by transplanting sensitized skin samples that retained their patch test-positive reactivity to iodoform and ringworm fungus (trichophyton) to normal recipients.¹⁶

Against this background of pioneering observations and studies, the patch test was introduced to dermatologic medicine. It would soon be evident that the simple application of a test contactant retained under a cloth patch had built-in risks for adverse reactions. Low's self experiments with *Primula* foreshadowed the potential for induced sensitizations of the tested area.¹⁸ Provoked constitutional reactions duplicated the experience of Jadassohn's patient, even when associated with a negative patch test.¹⁴⁶ Other reported hazards were the lighting up of healed, previously sensitized sites, exacerbation of quiescent lesions, and generalized spread of the localized eruption.⁶⁶ Additionally, there were unexplained phenomena of unique interest. The "crazy back" (currently known as the *excited skin syndrome*) referred to the effect of one strongly positive test reaction that produced a state of wide skin hyper-reactivity with resultant positive reactions at other unrelated patch test sites⁸⁷ (when first described, patch tests were placed on the back). The manifestation of the Koebner phenomenon occurred when reaction of a patch test contactant reproduced a lesion of psoriasis or lichen planus in a patient primarily affected by one of those dermatoses.

The finding that atopic and eczematous delayed sensitization to a common antigen in the same individual could coexist was demonstrated in furriers with allergic rhinitis, asthma, and contact dermatitis from paraphenylene diamine dye¹⁰⁴ and in medical personnel with hand dermatitis and asthma caused by exposure to neoarsphenamine.¹⁸⁴ As early as 1831, Elliotson in England attributed eczema on the hands of a patient carrying bundles of flowering grass to pollen contact.⁶³ One hundred years later in 1931, intracutaneous and patch tests implicated two different pollen components responsible for coexistence of the two distinctly different allergic mechanisms and disorders in the same patient: the oil fraction for seasonal (contact) dermatitis and the water soluble fraction as the atopen for seasonal allergic rhinitis.²³

A technique was described for passive transfer of blister fluid from a naturally occurring lesion of contact dermatitis or from a cantharides-induced blister (Urbach-Koenigstein method).¹⁷² Intracutaneously injected blister fluid did not reproduce the lesion but did confer demonstrable patch test positive contact sensitization and was interpreted as a cell-mediated function.

Through the medium of patch tests to provide determinative information, explanations were provided for two additional skin sensitization

phenomena. (1) The property of only some simple inorganic metal (e.g., nickel, cobalt, chromium) and low-molecular-weight organic compounds to act as cutaneous sensitizers depended on haptenic capability of combining with skin proteins to serve as contact allergens.⁶² (2) The mechanism responsible for skin eruptions evoked by handling specific types of drugs, which puzzlingly gave negative contact tests (particularly represented by sulfanilamide and chloropromazine), was elucidated by adding irradiation to the patch tests⁷⁹ (models for the study of chemical photosensitization and phototoxicity).

With his introduction of the concept of physical allergy, Duke described adverse reaction to physical agents (e.g., urticaria and dermatitis from natural sunlight and trial exposures to light from nitrogen, carbon arc, and mercury quartz lamps, and cold-induced angiodemia).⁵⁷ Skin contact lent itself to testing the phenomena from light sources of varying wave lengths and application of ice, respectively.

Jaddasohn's⁸⁶ and Bloch's¹⁶ introduction and development of the patch test provided insight on a skin test response that differed in timing and presentation of the immediate hypersensitivity reaction. Resultant appreciation of the delayed hypersensitivity response provided investigational leads for defining cell-mediated immunity.

SUMMARY

An overview of the first 25 years in the history of skin testing and its role in allergy's evolving place in clinical medicine was summed up by one of the method's foremost pioneers, M. Walzer:

"The skin test has been the most important single influence in the development of the subject of allergy . . . In the early years, attention concentrated on the improvement of the testing technics and of the materials employed for this purpose. This led to an overemphasis on the importance of this diagnostic procedure, so that, for a long time, progress in the field of allergy depended almost entirely upon development of the skin test. The failure to obtain positive cutaneous reactions in all forms of allergy was attributed to an inadequacy of testing materials and technics, and it was generally anticipated that the etiologic factors in all clinical forms of hypersensitiveness would eventually be revealed by improvements in testing methods. After more than a quarter of a century it has become apparent that these earlier hopes and prognostications for the future of skin testing were too optimistic . . . the fact that skin testing has not turned out to be a simple and completely reliable technic does not detract from the fact that when it is intelligently and skillfully performed, it remains the most effective diagnostic procedure in reaginic allergic disorders."¹⁹⁹

Outside the initially small arena of pioneering physicians who shared the experience of developing the materials and methods to give

allergy an evolving identity (those who understood and appreciated Walzer's reference to skin testing "skillfully and intellectually performed"), there was the larger world of practitioners whose sophistication in the modality left much to be desired. There were still those whom Clarke spoke of in his 1931 presidential address. "[He who] should be taught that his unskilled efforts to utilize the expensive materials sold him by detail men are not likely to be of benefit to his patient or of profit to himself . . . [who] should be told [of] the limitations . . . [and the] possibilities, and should be encouraged to cooperate to work in cooperation with the specialist in the clinic."³⁰ If education was to be the answer, coincident with the timing of Walzer's appraisal, the need had gotten underway.

The first step would be found in the wide scope of the large, almost encyclopedic, referenced coverage of the books that, following the standards set by the 1931 classic *Asthma and Hay Fever in Theory and Practice*, had become available through major publishing houses. Several spelled-out aspects of skin testing supplemented those for which Walzer's had eruditely set the stage¹⁹⁶. Urbach's and Gottlieb's *Allergy*, 2nd edition,¹⁷¹ Vaughan's and Black's *Practice of Allergy*,¹⁸² and the informingly comprehensive texts geared to the clinical needs of medical practitioners: Feinberg's *Allergy in Practice*⁶⁷ and Cooke's *Allergy in Theory and Practice*.⁴⁵

Because the interpretations and findings of investigational studies and case reports in large measure relied on skin test protocols, current journal literature continuously contributed new and updated information to the educational objective. To supplement the limited coverage and circulation of the bimonthly issued *Journal of Allergy*—the official organ of the American Academy of Allergy that predominately reached the organization's membership—the journal added an important regular feature in 1944. Allergy Abstracts was undertaken by alumni resident physicians of the Brooklyn Jewish Hospital allergy service, and its design incorporated reviews of the relevant world scientific and professional periodicals.

From time to time popular journals of general medicine carried relevant articles, particularly the *American Journal of the Medical Sciences* and the *Journal of Laboratory and Clinical Medicine* through the attention of their pioneer physician and allergist editors, Richard Kern and Warren Vaughan, respectively. Especially important to the interests and needs of practicing physicians and clinical allergists who were attracted to the objectives and programs of the American College of Allergy was the 1943 launching of the College's bimonthly journal, the *Annals of Allergy*.

An enlarging network of university medical center- and hospital-based allergy clinics founded by staff members, originally seeded from the first clinics in New York and Boston, provided opportunities for active training associations, such as the chance to observe and actively participate in the care of patients with asthma and atopic disorders and to learn the essentials of diagnostic techniques. Although not a mechanism for official control (such as licensing by state boards), the national professional allergy societies were able to help the cause by putting

stamps of approval on allergy clinics who sought such designation. Along with dealing only with facilities based in American Medical Association-approved hospitals that offered adequate treatment, diagnostic and consultant facilities, the joint Committee on Standardization of the SSAAC and ASA set requirements for "adequate skin testing materials by scratch (and/or) intracutaneous methods."³⁶ Members of the American Academy of Allergy (AAA) (founded by amalgamation of the SSAAC and the ASC in 1944) would have additional opportunities for developing diagnostic testing expertise by participating in collaborative, uniform protocols of the AAA's Research Council.³⁶

For those physicians for whom clinic voluntary staff and adjunct appointments and hands-on experiences were not available, there were still opportunities for interactive participation in outreach courses and attendance at lectures. In 1947, the same year as Walzer's appraisal,¹⁹⁹ the AAA Committee on Education had set up the first of its offerings for week-long, interactive, orientation courses in clinical allergy at University Medical Centers with invited faculties. The program basics included presentations and discussions of allergenic extracts and principles and techniques of skin testing.

A major development occurred in 1957 when within the structure of the National Institutes of Health (NIH), the National Institute of Allergy and Infectious Diseases (NIAID) evolved from the National Microbiological Institute (NMI). Added Federal support was thereby focused on allergic and immunologic research and training. One of the earliest studies to be undertaken in NIAID's Laboratory of Immunology was reminiscent of a statement of the AASACI and SSA joint Committee on Standardization at the time of its establishment in the early 1930s: belief that "the question of lack of correlation between skin test results and the expression of allergic manifestations was noted in too many cases under study . . . might one day be answered when etiologically responsible components of allergenic extracts were isolated and purified in the same manner that Avery had accomplished for the polysaccharide fraction of the pneumococcus."³⁶ Jules Freund, Chief of the Laboratory of Immunology and a foremost immunologist (who developed "Freund's [immunizing] adjuvant"), arranged for a 3-year collaborative project between a junior staff member, Wilton Vannier, and Dan Campbell of the California Institute of Technology faculty. Their project centered on the isolation and characterization of the allergenic fraction of house dust.

Although the Vannier-Campbell sophisticated immunochemical study met its objective,¹⁷⁶ the recovered fraction did not find its way to adoption by clinical allergists. Campbell again was called on in 1959 to establish and lead an NIAID-supported, extramural, collaborative research endeavor (Committee on the Standardization of Allergens) for the isolation and characterization of ragweed pollen allergen. From the studies of the 3-year research grant involving three component subcommittees on chemistry, laboratory animal models, and human-subject testing (recollection of the author [SCG] as a working subcommit-

tee member), short-ragweed major-fraction antigen E (isolated by T.P. King) was announced to the clinical community.

Ironically, the Standardization Committee's anticipated goal reaching fruition was realized only incompletely. Successful isolation was accomplished, but "answering the question of lack of correlation of skin test results" was not inherent in the interpretation and application of the research program's results. Although antigen E showed strong skin reactivity in all patients, the similar reactivity of minor fractions K (also in all subjects tested) and Ra3 and Ra5 in lesser numbers could not be discounted.⁹³ Pertinent to considering indicative test coverage, results of immunotherapy with antigen E were similar to those with whole extract.¹¹⁵ One century after Blackley produced a whealing response to grass pollen rubbed into an abraded area of his arm,¹⁵ it was back to the future.

The introduction and involvement of basic medical scientists into hypersensitivity research (encouraged, supported, and facilitated by NIAID research grant programs) was a critical factor in the changing shape of allergy's evolution as a discipline. Principles and practices that had been based solely on observation, description, and interpretation of phenomena gave way to translated applicable findings from studies in relevant aspects of immunochemistry, immunobiology, genetics, immunopathology, pharmacology, and molecular biology. All the more to be appreciated are the contributions of investigative studies from an era that, lacking modern-day sophisticated technology, were derived solely from application of motivation and qualities of intellectual curiosity, imagination, inventiveness, and initiative.

Meritorious examples are seen among those investigators and clinicians who found answers in the use and interpretations of skin tests. Rackemann's definition of skin test-negative (intrinsic) asthma¹³¹ paved the way for searches for etiologic factors (e.g., inflammation, infection, β -adrenergic blockade). Vallery-Radot's identification of a skin test-negative "hay fever"-like syndrome¹⁷³ led to recognition of sympathetic nervous system and endocrine influences in the pathogenesis of nonallergic rhinitis. Finding morphine and codeine's property of nonspecific induction of whealing that introduced the fact of histamine-liberating agents¹²³ can be viewed as a definitive step in discovery of mast-cell function. Recognition of timed eosinophil cellular responses in immediate hypersensitivity skin test reactions¹³⁶ foreshadowed the understanding of delayed asthmatic expressions. Revelations of blocking antibody and the P-K method of monitoring skin test reactivity provided the investigative approach that determined the reagin-diminishing effects of long-term immunotherapy.¹⁵¹

Since the skin test was first introduced to medical practice a few years short of a century ago, the physician oriented to allergic diseases and asthma has been presented with the benefit and advantages of a remarkable panorama of research and clinical advances. Not the least of these benefits is the technology of diagnostic *in vitro* immunoassays. Equally noteworthy is the observation that "skin tests [are] the original

[and] still most widely used method for demonstrating IgE antibody in the United States."²¹²

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APPENDIX 1

Development of Diagnostic Skin Tests for Infectious Diseases,* 1897-1944¹

Skin Test							
Date†	Disease (Agent)	Name§	• Test Reagent	Reaction		Serology	Note
				IMD	DEL		
1798	Smallpox (v)	Revaccination	Lymph from cow pox (vaccinia) lesion	+	+		2
1891	Tuberculosis (b) (<i>Mycobacterium tuberculosis</i>)	Tuberculin (Koch) Tuberculin modifications: Von Pirquet Calmette Moro Mantoux Schick Casoni	<i>M. tuberculosis</i> culture filtrate <i>M. tuberculosis</i> culture filtrate <i>M. tuberculosis</i> culture filtrate <i>M. tuberculosis</i> culture filtrate <i>M. tuberculosis</i> culture filtrate <i>C. diphtheriae</i> toxin Killed gonococci Hydatid cyst fluid		+	+	3
1907							4
1907							5
1908							6
1908							7
1908	Diphtheria (<i>Corynebacterium diphtheriae</i>)						8
1909	Gonorrhea (b) (<i>Neisseria gonorrhoeae</i>)				+	+	9
1911	Hydatid disease (h) (<i>Echinococcus granulosus</i>)			+	+	+	10
1911	Syphilis (b) (<i>treponema pallidum</i>)	Luetin	Heat-killed-cultured <i>T. pallidum</i> (extract)		+	+	11
1912	Gonorrhea (b) (<i>Neisseria gonorrhoeae</i>)		Killed gonococci		+		12
1913	Chancroid (b) (<i>Haemophilus ducreyi</i>)	Ito-Reenstierna	Ducrey's bacillus vaccine		+		13
1918	Scarlet fever (b) (<i>Streptococcus hemolyticus</i>)	Schultz-Charlton	Streptococcal antiserum	+			14
1922	Brucellosis (b) (<i>Brucella melitensis</i> and <i>Br. Abortus</i>)	Brucellin	Culture filtrate of <i>Br. melitensis</i> or <i>abortus</i>		+	+	15
1922	Ascariasis (h) (<i>Ascaris lumbricoides</i>)	Ransom	Helminth body fluid or extract <i>ascaris</i>		+	+	16
1923	Smallpox (v)	Tiéche	Sterilized variola pustule	+			17
1924	Coccidioidomycosis (f) (<i>Coccidioides immitis</i>)	Coccidioidin	<i>C. immitis</i> culture filtrate		+	+	18
1924	Scarlet fever (b) (<i>Streptococcus hemolyticus</i>)	Dick	Streptococcal erythrogenic toxin		+	+	19

Table continued on following page

Skin Test

Date†	Disease (Agent)	Name§	Test Reagent	Reaction		Serology	Note
				IMD	DEL		
1924	Aspergillosis (f) (<i>Aspergillus fumigatus</i>)	Frei	Aspergillus extract	+	+		20
1925	Lymphogranuloma (lymphopathia) venereum (b) (<i>Chlamydia trachomatis</i>)	Reverse Frei	Fresh bubo pus		+	+	21
1925	Erysipelas (b) (<i>Streptococcus erysipelatis</i>)		Heat-treated bubo pus		+		22
1926	(<i>Streptococcus haemolyticus</i> A)	Schröpl	Streptococcal erysipelatis soluble, sterile toxin		+	+	23
1926	Oxyuriasis (Pinworm) (h) (<i>Oxyuris vermicularis</i>)		Alcoholic Oxyuris extract		+		24
1926	Leishmaniasis (p) (<i>Leishmania brasiliensis</i>)	Montenegro	Leishmania (whole organism)		+	+	25
1926	Strongyloidiasis (h) (<i>Strongyloides stercoralis</i>)	Fülleborn	S. <i>stercoralis</i> dried powdered extract	+			26
1927	Schistosomiasis (h) (<i>Schistosomiasis spindalis</i>)		Saline extract of dried infected liver of snails	+	+	+	27
1927	Diphtheria (b) (<i>Corynebacterium diphtheriae</i>)	Moloney	Fluid diphtheria toxoid		+		28
1928	Trichinosis (h) (<i>Trichinella spiralis</i>)	Trichinella	Saline extract of dried powdered <i>T. spiralis</i> larvae	+	+	+	29
1928	Hookworm (h) (<i>Ancylostoma duodenale</i>) (<i>Necator americanus</i>)		Extract of dried, pulverized <i>A. duodenale</i>	+			30
1929	Pneumococcal pneumonia (b) (<i>Diplococcus pneumoniae</i>)	Francis	Type-specific capsular polysaccharide	+		+	31
1929	Smallpox (v)	Hooker	<i>D. pneumoniae</i> protein		+		32
1929	Moniliasis (f) (<i>Monilia albicans</i>)	Oidionycin	Killed vaccinia virus		+		33
1930	Gonorrhea (b) (<i>Neisseria gonorrhoeae</i>)		Monilial culture filtrate		+		34
1930	Filariasis (h) (<i>Dirofilaria immitis</i>)	Filarial	Gonococcal carbohydrate fraction		+		35
1930	Trichophytosis (f) (<i>Trichophyton purpureum</i>)	Trichophyton	<i>D. immitis</i> saline extract	+			36
			<i>T. purpureum</i> culture filtrate		+		37

1930	Trichophytosis (f) (<i>Trichophyton purpureum</i>)	Trichophytin	<i>T. purpureum</i> culture filtrate	+	38
1932	Trichophytosis (f) (<i>Trichophyton purpureum</i>)	Trichophytin	Trichophytin polysaccharide	+	39
1932	Tularemia (b) (<i>Pasteurella tularensis</i>)	Foshay	Detoxified <i>P. tularensis</i> /protein	+	40
1933	Tuberculosis (b) (<i>Mycobacterium tuberculosis</i>)	Tuberculin modification: Wolf	<i>M. tuberculosis</i> culture filtrate	+	41
1934	Gonorrhea (b) (<i>Neisseria gonorrhoeae</i>)		Gonargin (gonococcal polyvalent vaccine) and compligon (gonococcal toxin)	+	42
1934	Moniliasis (f) (<i>Monilia albicans</i>)	Oidomycin	Monilia culture filtrate	+	43
1935	Tularemia (b) (<i>Pasteurella tularensis</i>)	Foshay	Goat anti- <i>P. tularensis</i> antiserum	+	44
1935	Staphylococcus infections (b) (<i>Staphylococcus aureus haemolyticus</i>)		Type A staphylococcus bacterial protein	+	45
1935	Leptosy (b) (<i>Mycobacterium leprae</i>)				
	Tuberculoid	Lepromin	Leptotous skin nodules or <i>M. Leprae</i>		46
	Lepromatous	Lepromin	heat-killed suspension from <i>M. Leprae</i> -infected armadillos	+	47
1935	Gonorrhea (b) (<i>Neisseria gonorrhoeae</i>)		Compligon		48
1936	Gonorrhea (b) (<i>Neisseria gonorrhoeae</i>)		Gonococcal culture filtrate	+	49
1936	Tularemia (b) (<i>Pasteurella tularensis</i>)	Foshay	<i>P. tularensis</i> polysaccharide	+	50
1936	Staphylococcus infections (b) (<i>Staphylococcus aureus haemolyticus</i>)		Staphylococin type A carbohydrate	+	51
1936	Staphylococcus infections (b) (<i>Staphylococcus aureus haemolyticus</i>)		Staphylococin toxin and toxoid	+	52
1936	Blastomycosis (f) (<i>Blastomyces dermatitidis</i>)	Blastomycin	Heat-killed blastomycetes	+	53
1939	Brucellosis (b) (<i>Brucella melitensis</i>)		Brucella nucleoprotein		54
1940	Pertussis (b) (<i>Haemophilus [Bordetella] pertussis</i>)	Brucellergin	Detoxified <i>H. pertussis</i> bacterial agglutininogen antigen	+	55
1940	Pertussis (b) (<i>Haemophilus [Bordetella] pertussis</i>)		<i>H. pertussis</i> endotoxin	+	56
1941	Gonorrhea (b) (<i>Neisseria gonorrhoeae</i>)		Gonococcal culture filtrate	+	57
1941	Influenza bacillus infection (b) (<i>Haemophilus influenza</i> , type b)		<i>H. influenza</i> type specific polysaccharide	+	58

Table continued on following page

Skin Test

Date†	Disease (Agent)	Name§	Test Reagent	Reaction		Serology	Note
				IMD	DEL		
1943	Mumps (v)	Enders	Heat-inactivated suspension of mumps-infected monkey parotid gland		+		59
1944	Filariasis (h) (<i>Dirofilaria immitis</i>)	Filarial	Filarial saline extract	+	+		60
1944	Influenza (v)		Allantoic fluid of egg cultured influenza virus A or B		+		61
1941	Sarcoidosis ⁶² (Boeck's sarcoid)	Tuberculin Kviem antigen ⁶⁴				Unique reaction ⁶⁵	63 66

*Indicative of previous or present exposure.

†Date described.

§Specific name if given. Cutaneous (scratch) or intracutaneous/intradermal method unless otherwise indicated.

||Associated humoral antibody

IMD = immediate, 5-15 minutes, indicative of sensitization; DEL = delayed, 24-48 hours, indicative of humoral or cell mediated immunity; v = viral; b = bacterial; h = helminthic; p = protozoal

Notes:

1. Evolved from studies of infectious diseases that demonstrated development of interrelated, interdependent sensitization and immune responses (Topley WWC, Wilson GS: Principles of Bacteriology and Immunity, ed 2, Baltimore, Wood, 1936; Rich AH: Acta Pediat 16:1, 1933).
2. Response to (nontake) revaccination (Jenner E: An Inquiry Into the Causes and Effects of the Variolae Vaccinae. London, Sampson Low, 1798); variola-vaccinia shared antigenicity; not a designed skin test; sensitization and immune reaction.
3. Subcutaneous injection; diagnostic of infection (Koch R: Dtsch Med Wschr 17:101, 1891).
4. Abrasion (Von Pirquet C: Berl Klin Wschr 48:644, 1907).
5. Conjunctival (Calmette L: C R Hebd Seances Acad Sci 144:1324, 1970).
6. Percutaneous (More E: Münch Med Wschr 55:216, 1908).
7. Intradermal (Mantoux C: C R Acad Sci (Paris) 147:355, 1908).
8. Positive reaction diagnostic of susceptibility to infection; negative reaction indicative of sufficient antitoxin for immunity; positive (pseudoreaction) with parallel reaction of heat-inactivated control indicative of both immunity and allergy to a component (Schick B: Münch Med Wschr 55:504, 1908).
9. Subsequent evaluations demonstrated that skin tests with gonococcal organisms did not have practical diagnostic value (Bruck C: Dtsch Med Wschr 35:470, 1909).
10. Diagnostic of hydatid cyst (Casati T: Folia Clin Microscopia 4:5, 1911).

11. Diagnostic of infection (Noguchi H: *J Exp Med* 14:557, 1911).
12. See note 9 (Irons EE: *J Infect Dis* 11:77, 1912).
13. Positive tests persist after cure (Ito T: *Acta Dermat-Veneriol* 116:341, 1913).
14. Neutralizes erythrogenic toxin in skin (blanchung), indicative of presence of antitoxin and diagnosis of scarlatinal syndrome (Schultz W, Charlton W: *Z Kin Ber Heilk* 17:328, 1918).
15. Diagnostic of infection; may remain positive for many years (Burnet E: *Arch Inst Pasteur Afrique Nord* 2:187, 1922).
16. Diagnostic of ascaris infection and sensitization (Ransom B: *Parasitol* 8:42, 1922); cross-reactive with *Trichina*.
17. Transmitted to vaccinated recipient (or guinea pig substitute); positive reaction diagnostic of donor's infection with variola (Tiéche M: *Schweiz Med Wchnschr* 53:448, 1923).
18. Conversion to positive reaction is diagnostic of infection; cross-reactivity with histoplasmosis and blastomycosis; overwhelming infection effects anergy (Davis DS: *Arch Dermat and Syph* 9:577, 1924).
19. Positive reaction diagnostic of susceptibility; negative reactions indicative of antitoxic immunity (Dick GF, Dick GR: *J Amer Med Ass* 82:264, 1924).
20. Positive reaction diagnostic of role of aspergillus as a respiratory allergen or pathogen in aspergillosis (Storm Van Leeuwen W: *Proc Roy Soc Med* 17:19, 1924).
21. Diagnostic of infection; may remain positive for life; repeated tests induce skin sensitivity in absence of disease. Cross-reactivity with psittacosis and trichoma (Frei W: *Klin Wschr* 4:2148, 1925).
22. Heated bupo pus injected into pre-Frei positive recipient, positive reaction diagnostic of donor's infection with *L. venereum*.
23. Positive reaction diagnostic of susceptibility; negative skin test within few days after onset indicative of antitoxin production (Birkhaug KE: *Bull Johns Hopkins Hosp* 36:248, 1925).
24. Diagnostic of infection; positive results are useful as a guide for searches in areas in which infection is not prevalent (Schrüpl E: *Deutsch Med Woch* 52:1508, 1926).
25. Diagnostic of infection (Montenegro J: *Arch Derm Syph* 13:187, 1926).
26. Diagnostic of specific infection; in skin sensitive reactors; may share antigenicity with *Ascaris* and *Trichinae* (Fülleborn: *Arch F Schiffs and Tropenhygiene* 30:732, 1926).
27. Diagnostic of infection (Fairley NH, Williams FE: *Med J Aust* 2:818, 1927).
28. Intradermal injection of control-fluid diptheria toxoid produces accentuated pseudoreaction to serve as a Schick control when usual heat inactivated toxoid control does not (Maloney PJ, Frasier CJ: *Am J Pub Health* 17:1027, 1927).
29. Immediate hypersensitivity reaction diagnostic of infection may persist for many years after acute symptomatic stage; fulminating trichinosis may effect negative, anergic reaction; shares antigenicity with *Ascaris* (Bachman GW: *J Prev Med* 2:35, 1928).
30. Diagnostic of active (possibly also previous) infection and sensitization (Coventry FA, Taliaferro WH: *J Prevent Med* 2:273, 1928).
31. Immediate positive skin test is of favorable prognostic significance; persists after recovery; negative tests foreshadows nonrecovery (Tillet WS, Francis T: *J Exp Med* 50:687, 1929).
32. Delayed positive skin test with protein is species; but not type specific (Tillet WS, Francis T: *Idem*).
33. Reaction indicative of degree of subject's resistance to smallpox; negative reaction indicates susceptibility; skin test replaces need to revaccinate to determine vaccinoid (accelerated) reaction as indication of partial immunity (Hooker S: *J Inf Dis* 45:255, 1929).
34. Patch test eczematous response (Ramel E: *Rev Mé Dela Suisse Rom* 49:887, 1929).
35. See note 56 (Casper W: *Klin Wschr* 9:2154, 1930).
36. Immediate reaction indicative of exposure to infection, but not activity of disease (Taliaferro WH, Hoffman WA: *J Prev Med* 4:261, 1930).
37. Patch test, eczematous reaction more likely to be positive in eczematous "id" manifestations (Sulzberger MB, Lewis GM: *Arch Derm & Syph* 22:410, 1930). See note 39.

Footnote continued on following page

38. Urticarial immediate wheal reaction (Sulzberger MB, Kerr PS: *J Allergy* 2:11, 1930); most likely to be positive in erythrodermic "id" (Sulzberger MB, Rostenberg A, Goetz D: *JAMA* 108:2189, 1937). See note 39.
39. Tuberculin-type papular reaction; specific for Trichophyton, but of limited diagnostic value (Jaddassohn W, Schaaf F, Sulzberger MB: *Klin Wchnchr* 11:857, 1932).
41. Patch test (Wolff E: *Am Rev Tuberc* 27:308, 1933).
42. Reported as diagnostic aid; see note 56 (Heidenreich M: *Dermat Wchnchr* 99:1525, 1934).
43. Specific positive reaction, but of no practical diagnostic value (Kerr PS, Pascher F, Sulzberger MB: *J Allergy* 5:288, 1934).
44. Antibody reagent demonstrates presence of microbial antigen; test reaction specific (Foshay L: *J Allergy* 6:360, 1935).
45. For diagnosis of staphylocodermas; may be positive in healthy subjects and negative in present and past infections due to antitoxin formation (Julianne LA, Weighard CW: *J Exp Med* 62:11, 23, 31, 1935).
46. Positive reaction indicative of active or latent tuberculoïd infection (Bargheer P: *Muenchen Med Wchnchr* 52:56, 1935) (Mitsuda reaction originally described in 1919 [Mitsuda K: *Jap J Dermatol Urol* 19:697, 1919]).
47. Negative reaction indicative of anergy (Idem).
48. Clinical specificity demonstrated by passive transfer (P-K reaction) (Engle C, Vigilani MR: *Wien Klin Wchnchr* 48:48, 1935).
49. Reaction substantiated by negative control, see note 56 (Conrad CK: *New York State J Med* 36:1767, 1936).
50. See note 40 (Foshay L: *J Infect Dis* 59:330, 1936).
51. See note 45 (Julianne LA, Weighard CW: *J Exp Med* 64:149, 1936).
52. See note 45 (Sulzberger MB, Rubin G: *J Immunol* 30:386, 1936).
53. Of limited specificity in differentiation of comparable pulmonary infection (Martin DS, Smith DT: *J Lab Clin Med* 21:1289, 1936).
54. Delayed reaction diagnostic of infection; infrequent immediate urticarial reaction related to undetermined constituent of test reagent (Huddleson IF, Hardy AV, Debono JE, et al: *Brucellosis in Man and Animals*, New York, Commonwealth Fund, p 339, 1939).
55. Positive reaction indicative of immunity (Flosdorf EW, Kimball AC: *J Immunol* 39:475, 1940).
56. Positive reaction indicative of susceptibility to disease (Stearns LP: *Canad M A J* 42:525, 1940).
57. Usefulness not established (Corbus BC: *JAMA* 116:113, 1941).
58. Positive skin test diagnostic of production of specific antibody, prognostic of recovery (Dingle JH, Sideman LR: *Proc Soc Exp Biol Med* 46:34, 1941).
59. Negative reaction indicates potential susceptibility; of special value in diagnostic study of encephalitis without parotitis (Enders JF: *Ann Intern Med* 18:1015, 1943).
60. Predominately immediate whealing responses (see note 36); interpretation of occasional delayed papular responses undetermined (Bozicevich J, Hutter AM: *Am J Trop Med* 24:203, 1944).
61. Indicative of present or previous infection; positive skin test correlates with serology in children (but not in adults) with history of previous infection; sensitization may represent resistance to infection (Beveridge WTV, Burnet FM: *M J Australia* 1:85, 1944).
62. Currently recognized as an idiopathic disease; historically, following its clinical and histopathologic descriptions was considered a variant of tuberculosis (Schaumann J: *Études Bactériologique sur le Lupus Pernio et les Sarcoidoses Cutanées*. Stockholm, Norstedt & Soner, 1917) until 1953 following differentiation of anergic responses to testing agents other than tuberculin (Friou GJ: *Yale J Biol Med* 23:533, 1953).
63. Negative reaction denotes characteristic anergy.
64. Validated saline suspension of human sarcoid tissue, usually of splenic or lymph node origin (Kviem A: *Nord Med* 9:169, 1941).
65. Positive reaction, slowly enlarging granulomatous papule at intracutaneous injection site, reaching peak in 4-6 weeks.
66. Differentiates sarcoid from tuberculosis, leprosy, berylliosis, certain fungal infections, and local sarcoid reactions characterized by epithelioid cell tubercles.

APPENDIX 2

Skin Tests* in the Elucidation of the Phenomenon of Anergy,† 1911–1963: Demonstration of Negative DTHR§

Year	Disease	Test Antigen	Note
1911	Measles	Tuberculin	1
1914	Sarcoidosis	Tuberculin	2
1932	Hodgkin's Disease	Tuberculin	3
1934	Lepromatous Leprosy	Tuberculin	4
1934	Tuberculoid Leprosy	Tuberculin	5
1939	Crohn's Disease	Tuberculin	6
1941	Lepromatous Leprosy	Lepromin	7
1948	Coccidioidomycosis	Coccidioidin	8
1951	Tuberculosis	Tuberculin	9
1952	Sarcoidosis	Oidiomycin	10
		Trichophytin	
		Mumps	
1954	Sarcoidosis	Histoplasmin	11
		Pertussis agglutinin	
1956	Hodgkin's Disease	Microbial battery	12
1957	Sarcoidosis	Chemical sensitization	13
1963	Lepromatous and Tuberculoid Leprosy	<i>Candida albicans</i>	14

*To microbial products, except where chemical sensitization (introduced in 1941) is indicated.

†Original definition: transient specific loss of tuberculin hypersensitivity seen in patients with measles; subsequently expanded to describe absence or loss of ability to manifest delayed-type hypersensitivity reactions (DTHR) to a variety of DTH-effecting antigens.

§Negative tests provided definitive interpretations antigen specific.

Notes:

1. Prototype of viral infections responsible for effecting anergy (e.g., rubella, herpes simplex, influenza, infectious mononucleosis) (von Pirquet CE: Arch Intern Med 1:383, 1911).
2. Sarcoid originally considered a variant of tuberculosis. Negative DTHR differentiates sarcoidosis from some other hypersensitivity granulomatous disorders (e.g., hypersensitivity pneumonitis, silicosis, beryllium and zirconium induced processes) (Jaddassohn J, De Tuberkulide; Arch Dermatol Syphilgr (Wein) 119:10, 1914).
3. Parker F Jr, Jackson H Jr, Fitzhugh G: J Immunol 22:277, 1932.
4. Lepromatous patients demonstrated a low percentage of negative DTH tuberculin reactions (Wayson NE: Public Health Rep 19:1201, 1934); in another study, some patients had positive tuberculin DTH reactions (Lowe J, McNulty F: Lepr Rev 24:61, 1953).
5. Lower percentage of tuberculoid patients had negative tuberculin DTHR than normal controls (Wayson NE: Public Health Rep 19:1201, 1934).
6. Blackburn G, Hatfield G, Hunt AH: St. Bartholomew's Hosp Rep 72:181, 1939. Skin test studies in later years were contradictory; however in 50% of patients sensitization could not be induced (Jones JV, Housley J, Ashurst PM: Gut 10:52, 1969).
7. Dharmendra: Leprosy in India. 13:89, 1941.
8. Smith CE, Whiting EG, Baker EE, et al: Am Rev Tuberc 57:330, 1948. In overwhelming and disseminated coccidioidomycosis infection. Subsequently, some patients were found to have a broader loss of DTHR.
9. Overwhelming and acute disseminated tuberculous infection (Mascher W: Am Rev Tuberc 63:501, 1951).
10. First demonstrations of battery of nontuberculin, bacterial, viral, and fungal antigens (that produced positive DTHR by intracutaneous test injections in high proportion of normal individuals) to indicate broad spectrum of varied degrees of anergy in lepromatous leprosy patients. Over 90% of normal adults demonstrated at least one positive DTHR to a standard panel of antigens (e.g., tuberculin, mumps, streptokinase-streptodornase, candida, trichophytin) (electively also include dead histoplasmin, lepromin, atypical mycobacteria) (Friou GJ: Yale J Biol Med 24:533, 1952).
11. Sones M, Israel HL: Ann Intern Med 40:260, 1954.
12. Schrier WW, Roth A, Ostroff G, et al: Am J Med 20:94, 1956. Testing battery of microbial antigens (see note 10).
13. Epstein WL, Mayock RL: Proc Soc Exp Biol Med 96:786, 1957. Technique of normal receptivity to contact sensitization to challenge with and positive DTH patch test reaction to 2–4 dinitrochlorobenzene (DNCB) introduced by Rostenberg A, Kanof N: J Invest Derm 4:505, 1941.
14. First of studies to indicate relative anergy to nonmycobacterial antigens in leprosy (Buck AA, Hansclevier HF: Am J Hyg 77:305, 1963).