

increasing doses. The serum of those horses was dried, finely powdered and applied with a fine brush to the conjunctival mucous membrane or sniffed up the nose. This substance, called pollentine, proved extremely helpful to a very large number of hay fever patients and it was used on a large scale for many years, until at last the turning came, and the patients began to complain that they could not apply the powder any more without getting a serious reaction. It turned out that they had now become, in addition to their allergy to hay fever, allergic to horse serum. So that concluded that part of our work.

Before World War I, Prausnitz became head of the Hydrophobia Department of the Hygienic Institute at the University of Breslau, Germany. He describes the discovery for which he is most famous.

A few years after the war, one of my collaborators in the Hygienic Institute in Breslau, Dr Küstner, said to me that he was allergic to fish. Was I interested in the subject? I said, "Yes, very much indeed." We did some preliminary experiments with fish extract on him which showed him to be extremely sensitive. In fact, if he had the slightest trace of fish, sometimes without knowing that he had done so, he within a few minutes got asthma, urticaria, vomiting and diarrhea. An interesting paper by Ramirez had just appeared. A man had developed asthma after contact with horse dander late in life. Ramirez found that this man had just been transfused with the blood of a man suffering from asthma after inhalation of horse dander. That seemed to me to be a most interesting experiment, but I did not wish to become permanently allergic to fish. So I decided to carry out an experiment by injecting intradermally to myself various dilutions of Küstner's serum, and the following day, to inject each of those places, as well as control places in my skin with fish extract. The result proved that allergy to fish could be transmitted from the allergic person by his serum to healthy, normal people. But it didn't last; it was only a momentary, transitory, reaction.

Prausnitz and Küstner phenomenon: The P-K reaction

Discovery

Until the simple but clever experiment reported by Carl Prausnitz (1876-1963) and Heinz Küstner (1897-1963) in 1921,¹ neither an exact pathophysiologic mechanism nor the mediator responsible for evoking the hypersensitive immediate wheal-and-flare response had been elucidated. In their demonstration of blood serum transference of specific cutaneous reactivity were 2 unusual circumstances. First was Küstner's manifestation of clinical symptoms after eating fish, but only if cooked, and the development of a positive immediate skin test response to fish, but only if the extract had been preboiled. In the usual patterns of food sensitization, denaturation destroyed or diminished the antigenicity of a protein; in Küstner's case chemical alteration apparently created an allergenic fraction.*

The second related to the nature of Küstner's systemic reaction to fish. Its immediate onset and constitutional symptoms, including respiratory tract involvement, resembled typical sensitization and acute constitutional shock in the guinea pig. But unlike the experimental animal phenomenon, Küstner's serum was found to be devoid of precipitating, complement-binding, or antigen-neutralizing anaphylactic antibody and lacked the ability to passively sensitize guinea

pigs. Considering the possibility that Küstner was affected by an anaphylactic variant, they designed and conducted an investigative experiment targeting homologous species tissue, human skin.

Prausnitz's arm served as the recipient site of an intracutaneously injected sample of Küstner's serum. The later injection of a minute quantity of boiled fish extract into the targeted skin site evoked a typical and immediate wheal-and-flare response. Because evidence that the result did not have anything to do with the test recipient's own allergic state (Prausnitz had seasonal hay fever), they obtained identical results in repetition of the experiment with Küstner's serum transferred to healthy human test subjects. In considering the possibility of a cell-carried supersensitivity, they failed to transfer with a solution derived from a preparation of ground and scraped deep cutis and subcutis layers of skin. In other control subjects, attempted transfers with sera from donors who had positive skin test responses to horse serum and tuberculin were also negative.

At the same time, Küstner's inability to accept passive transfer of rye grass pollen hypersensitivity from Prausnitz's serum was puzzling. Putting the issue on a quantitative basis, they wondered whether success might have been possible if sera from more highly grass-sensitive donors had been available. The answer to that conundrum awaited later explanation.

Within the next 4 years, confirming reports by other investigators substantiated the Prausnitz-Küstner (P-K) experiment to be a valid original discovery. In 1923, passive transfer of immediate wheal-and-flare skin test reactivity to horse serum was demonstrated by de Besche⁴ with sera from horse-sensitive asthmatic patients and from subjects with hay fever from grass pollen, and with sera from subjects with hay fever by

*Similar uncommon experiences involving processed foods were subsequently reported: Duke's 1926 citation of a patient whose asthmatic reactions to peanuts occurred after eating only roasted and salted varieties,² and Rowe's 1931 observation on hypersensitiveness to cooked, but not raw, fruits.³

Freeman in 1924⁵ and by Coca and Grove in 1925.⁶ Far from observing just an isolated or unusual occurrence, Prausnitz and Küstner had uncovered a reproducible immune system phenomenon.

This classical P-K experiment is recognized as the starting point in the study of the nature of skin-sensitizing allergic antibodies and their assays. Applications of the passive transfer technique would prove to be a particularly useful diagnostic alternative to direct skin tests when needed and a valuable method for measuring sensitizing activity without subjecting a normal recipient to the risk of a systemic allergic reaction.

Characterization

In Coca and Grove's⁶ extended investigations on induction of passive transfer, 2 identified factors were particularly applicable to circumstances questioned in the original P-K experiment. First was the inability to transfer Prausnitz's reactivity to rye grass pollen extract. Coca and Grove found that not all tested subjects were uniformly susceptible to acceptance of passive transfer sensitization with sera from patients with pollen-induced hay fever. Perhaps Küstner fell within the 11% whose skins were found to be wholly nonreceptive to passive sensitization or the 5% who were only slightly receptive. Second, they also determined that the sensitizing power of a passively transferred serum, although unaffected by freezing and thawing, was distinctly lessened by preheating the sample for one-half hour at 56°C. In this connection Prausnitz was not aware of a protocol modification practiced by and apparently known only to his collaborator. As related by Frankland in the accompanying article in this Archives feature, it was only in later years that he (Prausnitz, then known as Giles) learned of the critical factor. As a presumed health-protective measure, Küstner treated the blood sample, awaiting transfer by boiling it for 10 minutes before self-administration. Little did Küstner then know that years later his protective caution would be shown to have a valid basis.

Lacking evidence of an immune mechanism for production of the serum factors responsible for positive skin test responses and P-K passive transfer of specific hypersensitiveness, Coca avoided classifying them as antibodies. Instead, he opted for the noncommittal designation of atopic reagins (Greek for reactive [substances]), an adaptation of the term then in use for the visualized and reacting serum element in the Wasserman reaction. With Grove, Coca then demonstrated unique properties of atopic reagins that clearly differentiated them from anaphylactic antibodies. Specifically, reagins (1) could be demonstrated only by their production of immediate wheal-and-flare tissue reactions both in direct and indirect (P-K) cutaneous tests, (2) were passively sensitized by the mechanism of transference and adherence to human tissue, (3) maintained transferred induced local

sensitization for periods of 6 weeks, (4) maintained *in vivo* local retention that allowed for repeated reactions with identical doses injected into the same demarcated intracutaneous sites, (5) lacked *in vitro* precipitating and complement binding properties, and (6) were unable to passively sensitize the guinea pig.⁶

In continuing study of differentiating properties, Levine and Coca established that the diagnostic reliability of a direct positive skin test response could be invariably validated by a correspondingly demonstrated P-K transfer reaction (in contrast to an irritant, false-positive, or nonspecific response to a histamine-liberating chemical). Furthermore, although the allergen in mixtures with corresponding reaginic sera before inoculation was capable of inhibiting the sensitizing power of the reagent, a reciprocal neutralizing action of reagent on allergen did not occur.⁷

In acceptance of the concept that followed, reagins were considered to be the sole property of blood content of individuals who, by virtue of hereditary predisposition, were subject to seasonal hay fever, perennial rhinitis, asthma, or infantile eczema (atopic dermatitis). The common denominator of this group of diseases was characterized by positive immediate cutaneous reactions to tests with causative allergens. Atopic reagins, products of natural occurrence and self-replication, *in vivo* were long lasting and persistently demonstrable by using the P-K technique of passive transfer. Continuing study, however, revealed deviations from this limited view with discovery of variants induced by artificial or extrinsic agents.

The variable development of passive serum transfer of local cutaneous hypersensitivity to some drugs, such as mercury, bismuth, salvarsan⁸ (possibly acting as haptens), insulin,⁹ liver extract,¹⁰ and horse serum (following administration of therapeutic antisera¹¹), could be definitively demonstrated, however infrequently. Unlike those P-K reactions that occurred as a consequence of development of hypersensitivity to naturally occurring allergens, such phenomena were characterized by finite or short-term durations up to several weeks or months. By contrast were nonatopic individuals who manifested immediate positive skin test responses to horse serum without accompanying P-K test reactivity after administration of horse-derived antisera. Lacking a reaginic basis, they were found to represent only acquired nonatopic skin hypersensitivity in healthy individuals and were without clinical significance.¹² A similar circumstance presented in infants who acquired benign skin sensitivity after being exposed to new foods on dietary addition.¹³ In rare exceptions the failure to demonstrate (subthreshold?) reagins to horse serum in a patient with an allergic hereditary background did not eliminate the risk of a constitutional reaction to administration of the foreign species antiserum.¹²

Although also variant, the serum antibodies demonstrated by Brunner in patients with *Ascaris* species

infections and in subjects who received injections of extracts of tissue-invasive helminths resembled the naturally occurring atopic skin-sensitizing reagins. They too were characterized by uniformly frequent production, long-term duration of positive wheal-erythema skin tests and P-K reactions, and ability to neutralize the corresponding allergens.¹⁴

Although P-K tests were most feasibly accomplished by means of a passive transfer of reagin-containing sera, the presence of reagins in other body fluids allowed for an alternative source for study, such as in nasal secretions¹⁵ and in colostrum of allergic mothers.¹⁶ To the opposite effect, the inability of reagins to pass membranes of the placenta¹⁷ and the choroid plexus¹⁸ negated comparable use of cord blood and cerebrospinal fluid, respectively.

Various modifications of the P-K testing procedure were used in the assay of reagins. In an experimental trial of reverse order of administration, passive transfer of allergen injected optimally 12 hours before reagent serum proved to be more sensitive than when given in conventional order.¹⁹ Other variations designed to assay absorption and timing included administration of challenging antigen from distant sites, such as when injected into the opposite arm,²⁰ insufflated into the nostrils,²¹ and introduced into the stomach.²²

Clinical relevance and diagnostic applications

Investigations of the P-K reaction and the nature of its mediating reagins uncovered and advanced knowledge on the phenomenon of immediate hypersensitivity and its disorders. In hay fever the degree of skin reactivity to a specific pollen was shown to be a direct quantitative representation of the corresponding reagent content of serum,²³ but a similar correlation was not found in asthma.²⁴ Although the degree of intracutaneous test response was directly proportional to the constitutional susceptibility of the patient for the injected allergen, it was unrelated to the severity of clinical manifestations.²⁵ Even positive direct and indirect test responses occurred in the absence of symptoms. A requirement for initial production of specific reagins was actual exposure to the corresponding allergen.²⁶ A related finding was that the identification of reagins, in the absence of a clinical history, could serve as a marker of potential sensitivity that often foreshadowed the development of symptoms.

Reagins that were identifiable by means of P-K demonstration were not likely to be found at all in other putative allergic disorders, such as angioedema, urticaria, migraine, and drug hypersensitivities, without a coexistent history of hay fever, asthma, or atopic eczema. Even in demonstrable skin test reactions associated with nonprotein drug reactions (eg, aspirin and quinine), P-K reactions were negative. However, in some instances, it was possible to elicit a positive

test reaction to an extract of the plant from which a drug was derived. Affinity of atopic reagins for, their honing to, and their retention in skin explained the persistence of positive skin test responses to horse proteins for several weeks in an inadvertently passively sensitized patient after subsidence of symptoms after receipt of an inadvertent blood transfusion from a horse-sensitive donor.²⁷

Following up observations on the ease, constancy, and reliability of passively transferring positive direct cutaneous reactions without sacrifice of specificity for the inciting antigens, M. Walzer and Kramer's investigations introduced the practicability of applying the P-K technique to clinical diagnosis.²⁸ Through extensive study and experience, Walzer designed and developed feasible applications for the use of practitioners and investigators. Until later years, when radioisotope labeling and RAST techniques would become available for *in vitro* techniques, dependence was placed on *in vivo* (P-K) identification, verification, and interpretation of the role of skin-sensitizing atopic reagins. For that purpose, Walzer set forth details of the necessary skills and guidelines that took into account a good number of carefully worked-out factors.²⁹ These pertained to the following: collection and sterility of sera; preparation of dilutions; placement of test sera and antigens on recipient sites; avoidance of exposure to strong allergens and intake of highly allergenic foods that could light up and deplete reagent-containing sensitized sites; adequate timing of intervals between serum transfers and challenges and between successive tests; avoidance or minimization of irritant tissue inflammation; and controls for and recognition of nonspecific determinants of refractory responses. Especially pertinent was appreciation that a positive P-K reaction indicated only that reagins for an allergen were present in the patient's serum and did not furnish proof of clinical sensitivity without the confirmatory correlation of symptom manifestations.

The P-K method was not a recommended substitute for routine direct testing but rather found to be especially useful when the direct method was deemed impossible, impractical, or inadvisable. Walzer's main indications for its use centered on the following conditions and circumstances²⁹:

1. abnormal skin conditions of the patient, such as (a) acute and chronic atopic eczemas; (b) ichthyotic skins and those scarred by constant scratching and inflammation; (c) urticaria; (d) dermatographism; (e) contagious skin infections, such as impetigo; (f) diffuse skin eruptions; and (g) hyperirritability of skin, especially in infants;
2. constant and severe asthma necessitating almost uninterrupted use of suppressive epinephrine;
3. extreme sensitivity, especially in infants and children, in which the possible development of severe constitutional reactions on direct testing posed a risk;

4. suspected hypersensitivity in infants and children too small or too ill to be subjected to a long series of tests;
5. antipathy on the part of the patient or relatives to direct tests;
6. inability of the patient to visit the physician or undergo a series of direct tests, either because of debility, inconvenience, or lack of time; and
7. desire or use to check the genuineness of an unusual number of positive skin reactions elicited by means of direct testing.

The P-K technique was also of particular value in surveys for food allergens. Challenges with suspected and controlled foods could be accomplished by test feedings in 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 test responses to specific foods. In such circumstances the causative allergen was viewed as a secondary derivative (ie, not acting in its primary state as ingested but rather as a split product of digestion or metabolism). In a reversal of the P-K procedure, A. Walzer demonstrated a method in which initial ingestion of a test food, later followed by intracutaneous passive transfer of test serum would elicit a spontaneous reaction at the test site.³⁰ Another variation of the P-K technique allowed for the patient to serve as the test subject of his or her own reagins. Named autopathetic transfer at intracutaneously prepared self skin sites, the patient's own (autogenous) transferred serum would react with allergens injected from remote areas.³¹

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.

A research tool

Application of the P-K phenomenon beyond diagnostic use and validating the identification of allergenic agents in patients' individual cases expanded into the research arena. Incorporation of passive transfer methodology was introduced into the protocols of investigations of a range of clinically relevant questions. Interpretations of data generated by means of indirect testing were important in evolving an understanding of the concept and mechanism of hypersensitiveness.

As a qualitative assay, surveys for identities of reagins could be made by using cross-challenges of passively sensitized sites with extracts of similar and different allergens. Exhaustion of a repeatedly tested site did not prevent additionally demonstrable local

responses to challenges of retained reagins with other test antigens. In a similar fashion relative concentrations of reagins in transferred serum samples could be comparatively estimated by determining points of their depletion or retention, with the latter determined by reaction at an exhausted site evoked by a stronger concentration of the identical test allergen. Advances in hypersensitivity research were facilitated and expedited in 1926 with Levine and Coca's introduction of technical modifications of the P-K reaction incorporating dilution and neutralization procedures.

In the dilution technique progressive dilutions of the sensitizing serum were passively transferred to demarcated recipient sites, and then each was subsequently tested with the identical lot and concentration of antigen. The highest dilution titer to induce a positive reaction reflected the activity and content of reagent. In studies developing this method, Levine and Coca demonstrated that during the early stages of injection to treatments with ragweed extract, levels of skin-sensitizing reagins actually increased.²⁵

The neutralization test began with mixing a fixed amount of sensitizing serum *in vitro* with graduated amounts of test antigen. After incubation at refrigeration temperature, the mixtures were passively transferred to multiple recipient sites. Reactions elicited by testing each site with an antigen of identical volume and concentration determined the relative amount of antigen that was taken up or had neutralized any or all of the skin-sensitizing antibody in each mixture. The result thus reflected the original reaginic titer.⁷ Through a first application of the Levine-Coca neutralization method, Cooke and associates³² were able to compare the behavior of serum samples taken before and after treatment injections of ragweed extract. This study led to their identification of a factor that had developed in the sera of treated patients and inhibited the action of reagent on allergen, the blocking antibody.

Although as entities reagins were not tangible substances, demonstration of their reactivity in the gamma globulin fraction of the blood paralleled the identical distribution of serum agglutinins and precipitins, an observation that contributed to their visualization as antibodies. Multiple and varied *in vitro* tests that sought to replace the P-K technique followed. Initial attempts to quantitate sensitizing antibodies by their putative agglutination of allergen-linked erythrocytes, collodion particles, or platelets, however, fell short. That reagent was actually being measured lacked firm evidence. *In vitro* results were not uniformly correlated with P-K detection, and injection treatments contributed to some extent to the development of high hemagglutination titers.³³

In 1967, the discovery and isolation of IgE introduced a new dimension and direction in the methodology of allergic disease research. Of interest was that pertinent to the identification and validation of

δE as the atopic reagin, the pioneering investigators used and depended on the P-K technique.³⁴ Until the opportunity the T. and K. Ishizakas' work presented for precise analysis and quantitation of hypersensitivity-mediating antibodies, indirect passive transfer tests had provided 4 decades' use of a reliable research tool.

Expediting the accumulation of new knowledge on the nature, activities, and roles of the atopic reagins enhanced opportunities to initiate early studies on the mechanism and differentiation of hypersensitivity phenomena and disorders. Important in the long list that ensued were those that dealt with hyposensitization treatments (isolation and questionable *in vivo* effects of blocking antibody and changes in reagin titers); immune nature of skin and allergic constitutional reactions; mechanisms of neonatal sensitization; shared cross-relationships and dominance of antigens; classes of allergens (commonalities and differences within groups of pollens, foods, molds, plant seeds, and animal species danders) and their practical applications to diagnosis and treatment; multiple antigenic components of allergens originally considered to be single entities (particularly house dust); effects of new drugs (particularly with antihistaminic properties and adrenergic influences) on production and levels of skin-sensitizing antibodies; absorption and distribution of allergenic agents; electrophoresis as an alternative to hyposensitization by injection; recognition of hypersensitivity manifestations of fungal skin infections and insect bites; identification of allergic potential of newly introduced natural and synthetic products and drugs; nature of the physical urticarias; and roles of incomplete and haptenic antigens.

A highly valued spin off of the P-K technique was the promotion of collaborative research facilitated by the feasibility of collecting, storing by means of deep freezing or lyophilization, and sharing of contributed high-titer reagenic sera. With the means to ship samples of common or special interest and specificity to geographically disparate investigators, Walzer and Siegel established the first model reagin bank at Brooklyn Jewish Hospital. Its formation got underway as a cataloged resource for collaborative studies undertaken under the sponsorship of the Research Council of the American Academy of Allergy.

Finis

Whatever value the P-K method might have had in diagnostic and investigational studies of allergic diseases was overshadowed in the mid-1960s by discovery of the hepatitis virus and its identification as a blood-borne, serum-transmissible infectious agent. The prior procedure of insuring safety through the use of sterile collection and storage techniques, Seitz filtration for microbial removal, and eliminating Wasserman-positive (or equivalent serum test for syphilis) samples could no longer suffice. The

American Academy of Allergy Brooklyn Jewish Hospital reagenic serum bank had no alternative to disposing of its unique stock and to ceasing operations.

P-K testing of necessity faded from popular and advantageous application in human clinical medicine. However, its principle as described by Prausnitz and Küstner provided continuing alternative research outlets in animal models, Ovary's extensive passive cutaneous anaphylaxis studies in laboratory sensitized rats and mice,³⁵ and the naturally occurring allergic dog.³⁶

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