ANTIBODIES OF ATOPY AND SERUM DISEASE IN MAN[1,2]

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The "immediate" class of antigen-antibody reaction in man is represented by atopy and by serum disease, a syndrome evoked by foreign sera, drugs such as penicillin and insulin, and insect venoms. Whereas atopy depends on skin-sensitizing antibodies called reagins, the other disorder is associated with reagins of perhaps a different quality and also with precipitins. Atopies are genetically oriented, appearing in certain families after natural exposures to such harmless substances as airborne pollens, while serum disease manifestations follow the abnormal exposure, through injection, of average individuals to unusual substances. Serum disease is often ushered in by a 7 to 24 day incubation period during which antibodies are being formed. Once these become available to residual antigen, a union initiates atopy-like urticaria and edemas, as well as other manifestations (fever, arthralgia, and adenopathy) that are not encountered in atopy. Reexposure leads to accelerated, and finally to immediate, atopic developments, such as hives, asthma, hay fever, and even shock. It is tempting to attribute this dual expression of the antigen-antibody reaction in serum disease to two different antibodies, reagins and precipitins. Not infrequently, still later contacts in serum disease and atopy may evoke no adverse signs, a tolerance having been acquired either spontaneously, in rare instances, or after specific immunization as in wasp venom allergy and pollen hay fever. Such tolerance may be referable to blocking antibody, which commonly coexists with reagin and is known to bind antigen *in vitro* or *in vivo* without insult to nearby cells—a virtue which, in addition to its superior avidity for antigen, should endow it with protective qualities.

Unfortunately for those who would isolate it, this 7S γ-globulin fails, despite its *in vitro* antigen-binding, to precipitate. Reagin has proven even more elusive to characterization, as its union requires prior fixation to a living cell. These shortcomings of the two antibodies have precluded their removal from the serum in the form of an antigen-antibody floccule which could later be dissociated and examined for antibody size, shape, charge, combining ratio, biologic role in quantitative terms, and for modifications that might be imposed on its activity by competitive antibodies, temperature, time, pH, etc. Techniques emanating from the schools of Heidelberger,

[1] The survey of the literature pertaining to this review was concluded July 1, 1965.

[2] The following abbreviations will be used: PCA (passive cutaneous anaphylaxis); SSA (skin-sensitizing activity).

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Landsteiner, Tiselius, Svedberg, Grabar, and others have provided such data for many experimental antibodies. Currently, scientists are not only concentrating fruitfully on the more difficult problem of the human antibody, but they are discovering analogues of human immune bodies in animals, thereby broadening the scope for experimentation and compensating to a degree for our present paucity of knowledge.

Before discussing such fundamental contributions of the recent past, passing mention will be made of a few devices which will aid the practical management of allergic patients. Substitutes have been proposed, for example, for the mineral oil constituent of Arlacel-petrolatum emulsions. [The latter have so retarded the release of incorporated allergen that a single, annual repository (1) can safely and effectively replace the long, conventional series of weekly injections.] One substitute consists of 2 per cent aluminum monostearate in peanut oil, which is showing promise in human recipients of influenza vaccine (2). Another substitute is a synthetic hydrocarbon (3) which is under pilot trial by the writer. So far, the safety and efficacy of about 100 repositories given in 1965 have equalled those of mineral oil emulsions employed in the past (1). Although pyridine-precipitation of pollen extracts is held to delay release, thereby facilitating the abbreviation of prophylaxis, the protein N content of these preparations must be determined prior to the introduction of pyridine, and there is no way to know how much activity has been discarded along with the pyridine supernate. Thus, what appears to be a heightening of the patient's tolerance for dosage may be illusory.

Atopy

The relationship between pollen and hay fever was recognized long before the antibody. The discovery of diphtheria antitoxin had inspired Richet (4) to seek other antitoxins; he stumbled by serendipity onto animal anaphylaxis (through prolonging a yachting trip at the expense of his injection schedule). Shortly, Noon (5) among others saw its analogue in human asthma and hay fever. Trying to reproduce the phenomenon of desensitization which was so spectacular in the passively sensitized animal, he induced tolerance in grass pollen allergy by means of a series of pollen injections. Although this firmly established hay fever as an anaphylactic disorder, ten years elapsed before Prausnitz & Küstner demonstrated the skin-sensitizing factor. Reassurance lasted only six years, however, as the desensitization concept exploded when an increase rather than a decrease was noted in humoral reagins after a protective series of injections (6). Again by serendipity, Europeans ran into the explanation when they encountered occasional reaginic sera which, instead of conferring, inhibited the PK transfer. Then, Barnard, using ragweed sera in Cooke's laboratory (7), observed that inhibition was limited to post-treatment specimens, permitting the assumption that a new, induced antibody must be involved. Next, the writer (8) induced a pollen-neutralizing factor in the blood of three

nonatopic volunteers through intensive parenteral treatment with extract. Later discovery of the factor's heat stability (9) enabled her to show the same antigen-binding quality in treated hay fever victims' sera, free from the erstwhile confusing influence of their heat-labile reagins. Not only could the blocking antibody, as it came to be called, unite with the common antigen in a preferential manner that deprived reagin of antigen, thereby averting the wheal-and-flare reaction, but it did so without inflammatory aftermath (9). Moreover, blocking antibody acted instantly and irreversibly *in vitro,* without the need for prior fixation to living tissue as is necessary for reagin. Seven persons ill with hay fever and asthma exhibited prompt and sustained remission, as well as diminished cutaneous and ophthalmic reactivity when transfused with strongly immune blood from pollen-treated nonatopics (10). Blood taken from them just beforehand and transfused into normal persons conferred all the attributes of pollen allergy on recipients (11). Not only this opposing role but other qualities have since differentiated blocking antibody from reagin: its rapid diffusion from a cutaneous site (9), its penetration of the placental barrier, its electrophoretic migration with γ- rather than with β-globulins (12), its sedimentation coefficient of 7S as contrasted to the more rapid, intermediate rate of reagins (13), its postponement of precipitation by rabbit antiragweed serum through competition for the common antigen (14), its resistance to thiols which inactivate reagins (15), and its capacity to sensitize the guinea pig for passive cutaneous anaphylaxis (16).

Despite the above evidence from transfusions, the clinical role of blocking antibody remains disputed. One cause of conflicting data has been the use of the patient's serum rather than a clinically participating organ, such as the eye, to estimate blocking antibody output. A second uncertainty is introduced by restricting the comparisons of clinical and immunological data to groups that are observed during a single year, rather than to individuals watched over a succession of years. Such longitudinal studies, in conjunction with conjunctival instillation tests as a clue to immunity, have improved the correlation (17, 18). An inescapable difficulty remains, however: the investigator's dependence on his patient to judge the efficacy of therapy during a two-month long season. Sorely needed is an objective index to clinical resistance. A promising model has now been found in wasp-sting anaphylaxis, where acquired immunity can quickly and unequivocally be plumbed through planned stinging by live insects, as will be developed below.

Technics for Detecting Reagins *in Vitro*

Successful procedures rest on the fact that reagins fix themselves to living cells. Vital white cells no doubt accounted for the histamine that was released by antigen from reaginic blood (19) and from rabbit blood cells after their incubation with human allergic serum (20). Shelley (21) enhanced this principle by examining the basophil cell for microscopic

degranulation, while Van Arsdel (22) is improving the reliability of the human leukocyte test by using strictly fresh or promptly frozen reaginic serum. [Osler (23), by the way, and Patterson (24) have demonstrated inhibition of this action by blocking antibody.]

The hemagglutination test, currently popular, is no longer considered to reflect reagins (25). *In vitro* binding of I^{131} tagged pollen antigen by pretreatment sera (26) probably rests on blocking antibody, which may afford an explanation, too, for the precipitin lines given by γ_2-globulin (27) and by tenfold concentrations of γ-globulins from pretreatment sera (28) when they were diffused in agar gel against pollen antigen. Augustin (29) doubts that reagins precipitate antigen. Arcs formed by γ_1- and γ_2-globulins during immunoelectrophoresis (30) with radioactive antigen and rabbit antihuman serum (27) may also have stemmed from blocking antibody present in sera from treated and untreated pollen-allergics. Although antigen-binding was noted also in β_2A- and occasionally β_2M-globulins, the assumption that reagins were responsible was made without biologic test for skin-sensitizing activity (SSA) and is therefore tenuous. Kopp reports (99) that the binding of radio-labeled ragweed and house dust antigens by human immunoglobulins is poorly correlated with their skin-sensitizing activity.

Ortiz (31) used immunoadsorbents such as cellulose, bentonite, and aluminum hydroxide gel to pick up ragweed antigens, then added reaginic serum. Subsequent search for adsorbed antibody protein by chemical assay, fluorescent antiglobulin technics, etc. gave no convincing evidence for reagin-antigen combination *in vitro*. This outcome is consistent with our own early efforts to detect test tube inactivation of pollen antigen by reagin (32). If it took place at all, union was so loose as to undergo ready dissociation in the presence of tissue-fixed reagins.

Isolation of the Antibodies of Atopic Serum

Since antigen-antibody precipitation fails to occur *in vitro*, extensive efforts have been made to fractionate allergic sera by chemical and physical methods. Separation of the blocking activity from the rest of the serum has been achieved by various means. Cann & Loveless (12), for example, detected this activity in the fastest and slowest migrating molecules of the γ-globulins, when the bleeding had been done early in the donor's acquisition of insulin-resistance, whereas all γ-globulin constituents were active in later specimens. Numerous studies by others have established the 7S, γ-globulin nature of such heat-stable, antigen-binding antibodies and of hemagglutinins (33).

In the case of reagins, however, mixed evidence exists as to their distribution in the serum proteins. Our own studies (12) found that SSA paralleled the β-globulin content of electrophoresis-convection fractions. Sehon, Harter & Rose (34), on the other hand, reported activity in γ_1- and γ_2-globulins, the central portion of the β-globulin peak being inert. Using

gradient ultracentrifuge, Rockey & Loveless (35) detected the SSA of wasp venom reaginic serum in fractions having a sedimentation coefficient intermediate between the 7S and 19S components, thereby confirming Rockey & Kunkel (13). In contrast to the SSA noted in 19S fractions by Gynes, Gordon & Sehon (33), neither SSA nor blocking activity was detected in our 19S preparation. Ion exchange chromatography on DEAE, first applied to reaginic sera by Humphrey & Porter, yielded blocking and hemagglutination antibodies in the first eluate, whereas SSA was spread over the next several eluates (36). Indeed, Augustin (37) effected a complete separation of reagins and hemagglutination antibodies in this way. It was her impression that reagins are 7S γ_1-globulins which tend to complex with various serum proteins and become dissociated during later fractionation procedures—which may explain some of the foregoing inconsistencies.

A suggestion of Augustin led Heremans & Vaerman (38) to prepare, through zinc sulfate precipitation, a β_2A-fraction, mixed with some γ-globulin, that exhibited SSA. Fireman, Vannier & Goodman (39) procured a similar fraction, IV, from each of three ragweed allergic sera by means of starch electrophoresis and chromatography on DEAE cellulose. Its strong SSA was unaffected when rabbit antiserum specific for human γ-globulin was added to eliminate its γ-globulin and leave only β_2A-protein. A control admixture of the fraction with normal rabbit serum also caused no loss of Prausnitz-Küstner activity. When, however, the original reaginic sera were absorbed with a unispecific anti-
power disappeared. Control "absorption" with normal sheep serum had no effect, despite the risk of inducing a state of site hyporesponsiveness (32) in human skin with foreign serum, especially if urticariogenic.

The popular thesis for β_2A-reaginicity must face the finding that a young man, who lacked all evidence of β_2A-globulin in his serum during repeated immunoelectrophoresis examinations, nevertheless produced reagins (40). Indeed, he not only exhibited them in his blood according to Prausnitz-Küstner tests but gave typical allergic responses in skin and conjunctiva, as well as classical adverse reactions to excessive doses of pollen extract. Furthermore, serum reagin output was enhanced by a booster stimulus, which also provoked blocking antibody synthesis. Instances of reagin production in two persons who may have lacked β_2A have also been encountered by others (41). Counterbalancing this situation is a description by Fireman, Boesman & Gitlin (42) of a woman with ataxia telangiectasis who lacked serum β_2A-globulins but had circulating reagins for milk proteins, without clinical allergy. Although SSA was associated with her 7S γ_2-globulins, it proved to be only partially destroyed by heat and remained fixed to skin for less than a day. These atypical qualities were reminiscent of those detailed by Terr & Bentz (43) for serum disease reagins encountered in three persons allergic to horse serum. Fractions derived by Sephadex gel filtration, DEAE cellulose chromatog-

raphy, and zone electrophoresis revealed no SSA in β_2A-globulins, while activity located in the γ_2-globulins withstood thiol treatment and heating which destroyed the β_2M-reagins. These data suggest that reagins formed in serum disease differ from those in atopy. Perhaps the foreign protein mixture, milk, evoked a serum disease type of reagin in Fireman's patient? Earlier analyses by Terr & Bentz (44) of ragweed-reaginic fractions prepared by gradient ultracentrifuge revealed an intermediate sedimentation coefficient for SSA and its association with β_2A-globulins. However, they commented that the β_2A-specificity did extend into the lighter 7S region in all four sera, with SSA in two of them. When rattlesnake venom stimulates reagin formation in handlers (45), activity is again related to β_2A-globulins and an intermediate sedimentation coefficient.

A theoretical complication has arisen for the Prausnitz-Küstner test applied to such preparations, in that normal β_2A-globulin prevents transference of the wheal-and-flare reaction, presumably through competition for the skin's fixation site (46). The smallest amount of purified, normal β_2A-protein that sufficed to inhibit was 2.5 μg (0.1 ml of 25 μg/ml). Since the low limit of detection for the double diffusion analyses lay between 60 and 120 μg of protein/ml, it is obvious that other serum constituents could have contaminated their β_2A-preparation without detection. However, no role could have been played by blocking antibody, had it by chance been possessed by the original normal serum, since it would have diffused away from the site (9) during the one to two days interval that elapsed between sensitization and challenge. It was interesting, in passing, to note that the powerful reaginic serum could transfer skin-sensitizing activity with as little as 0.16 μg of β-globulin (the writer's estimate). The corresponding figure for our (47) most active β-globulin enriched fraction was roughly comparable, amounting to 1 μg of β-protein. These do not differ strikingly from the 2.5 μg of normal β_2A-protein required for inhibition.

The inhibition phenomenon surrounding normal β_2A in human Prausnitz-Küstner studies is reminiscent of that encountered in the guinea pig during passive cutaneous anaphylaxis (PCA) experiments with homologous serum (48). Sensitization can be inhibited by an excess of γ_1-globulin that is not specific for the antigen to be used in challenging the site. Inhibition is attributed to competition of these globulins for the tissue fixation site required by the 7S γ_1-globulins that comprise the heat-stable anaphylactic antibodies. The site where attachment takes place on the cell wall has not been identified, probably because of the small amount of antibody needed for anaphylactic sensitization. Not only these antibodies but normal, 7S γ-globulins obtained from man or rabbit can affix themselves to guinea pig skin. If, in addition, they are first made to aggregate, through heating for example, they will per se evoke an immediate cutaneous reaction in the skin and fix C′ (49), both attributes apparently resting on dimerization of γ-globulin or of its piece III.

Terry (50) applied the reversed form of PCA to show that it was the

γ_{2b}- and γ_{2c}-globulins of human myeloma serum which fixed themselves to guinea pig skin, and not the γ_{2a}-globulin, the latter being inert and of different antigenic specificity. [It was of interest to recall at this point that our subject who fabricated reagins but no β_2A could nevertheless confer classical Prausnitz-Küstner transfer reactions to normal human skin (40).]

Other advances stemming from animal experimentation will be discussed after a challenging discovery in the field of human atopy has been presented. Connell & Sherman (51) have found that pollen-sensitive persons under long-term specific immunization tend to lose their clinical susceptibility and their reagins, according to Prausnitz-Küstner tests. Concurrently, the writer (52) presented independent data on 47 ragweed-allergic patients, half of them previously untreated, whose eye-instillation requirements for the minimal allergic response had been determined each May or June for as many as 15 years. Initially, they had all exhibited satisfactory seasonal refractoriness to atmospheric pollen after each of one to six annual repositories and an equal number of years on short, weekly courses of fluid antigen. Thereafter, they had unknowingly been switched to emulsified saline repositories as a prelude to observation with no injection at all. Despite their original clinical, serologic, cutaneous, and ophthalmic evidence of clear-cut pollen allergy, 28 could be listed as provisional cures by or before 1964, nine others being candidates who had still to undergo some years of simple observation. While this encouraging outcome accounts for three-quarters of a group originally responsive to specific therapy, there were ten members who lapsed after a first, second, or third yearly placebo emulsion. Some of these, however, consequent to recommencing specific therapy, achieved a clinical tolerance that persisted through years of placebo management. When the ophthalmic requirements were examined for the entire group, it became apparent that primary treatment provided a slight elevation in baseline that was maintained from June to June, or else gradually rose. It was unlikely that the blocking antibodies that were induced by pollen injections accounted for this, in view of that antibody's ephemeral nature (53), most of it disappearing within a few months after a swift booster stimulus. The findings of Connell & Sherman lend credence to the alternative explanation, reduced reagin output. These findings should encourage allergists to interrupt injection therapy after a few successful seasons.

Analogues of Atopy and Serum Disease in Animals

As implied above, animals produce antibodies that are analogous to human reagins and blocking antibodies (54), the anaphylactic ones attaching themselves to tissue and responding to the arrival of antigens by liberating vasoactive amines, such as histamine or 5-hydroxytryptamine, from mast cells. This induces a sudden increase in vascular permeability with consequent exudation of serum proteins into the tissue, and local or generalized anaphylaxis. Such antibodies in the guinea pig and mouse are in-

duced in sufficient amount to permit isolation and characterization. They are γ_1-globulins, have 7S sedimentation constants, are heat-stable, and distinct from β_2A-globulins. They do not maintain their attachment to the skin of the homologous species as long as does human reagin (55). Anaphylactic antibodies formed by dogs (56) and by rats (57) migrate as slow β-globulins. That of the rat resembles human reagin closely, in that it is heat-labile, is found in very low concentrations in the serum, sediments between the 7S and 19S globulins, and remains fixed to rat tissue for long periods (58). Thus, each animal produces a unique antibody responsible for anaphylaxis in its own species. The fact that it must fix firmly to tissue imposes a need for one to four hours to be allowed, depending on the amount of antibody and antigen as well as on the species, before a PCA reaction can be obtained (54); the counterpart of the Prausnitz-Küstner response in man can be evoked by antigen.

Efforts to transfer anaphylactic activity to other species will fail unless an antibody other than the type responsible for this phenomenon in the donor is employed. This implies that the receptors on the antibody that enable it to attach to tissue vary with the species. Nevertheless, antibodies of man, rabbit, monkey, dog (59), or mouse (55) give a PCA reaction in the guinea pig. This depends on the γ_2-globulin, the C′-fixing molecule which presumably possesses on its H chain the particular structure which characterizes the guinea pig γ_1-anaphylactic antibody. It will be recalled that it is the human 7S, γ_2-blocking antibody that provides the PCA reaction (16). Ovary, Barth & Fahey (60) have recently discovered that the PCA reaction in guinea pig skin is based on the 7S γ_{2a}-globulin but not on the γ_{2b}-globulin of the mouse, two subclasses which differ in antigenic composition. It has already been mentioned that human myeloma γ_{2a} is inert in the guinea pig (50).

As to the mechanism involved in triggering the release of vasoactive amines, the hypothesis of a simple bridging of two cell-bound antibodies by one molecule of antigen has received recent substantiation (61, 62); equal intensities of PCA response being given by equimolar concentrations of bi- or multivalent hapten, but not by equal weights of these conjugates (63). Testing solutions comprised of benzylpenicilloyl-polylysine (which can be prepared in widely different molecular sizes) should, therefore, be labeled according to molarity of the entire conjugate rather than the weight per liter of the conjugate. (It appeared that 20 to 100 lysine residues per molecule would be suitable for clinical diagnostic studies of penicillin allergy.)

Whereas the foregoing data for anaphylactic antibodies in animals have implications for reaginic man, the Arthus precipitin of animals has less well defined relationship to human serum disease. It is characterized (54), pathologically, by hemorrhagic necrosis of the skin one to four hours after injection of the antigen into a passively sensitized animal. Only a γ_2-globulin, C′-fixing homologous or heterologous antibody will prove ef-

fective in the guinea pig, rabbit, or rat, and only precipitating antibodies mediate it (64), not the γ_1 anaphylactic type. About 1000 times more of the antibody N is required for the minimal Arthus than for the minimal anaphylactic response. Complexing of antigen, antibody, and C′ occurs under the endothelial cells next to the basement membrane of small blood vessels. It is not this immune precipitate per se that damages the vessel, but leukocytes which phagocytize it and release proteolytic lysozymal enzymes. Platelets accumulate, thrombi form, the wall is destroyed, and hemorrhagic necrosis ensues. Unlike the evanescent anaphylactic changes, this damage is organic and requires slow healing (54). To the extent that precipitins hasten the elimination of foreign antigens from the body in serum disease, they might be considered teleological, but it must be remarked that any defensive role played by either anaphylactic or precipitating antibodies may be costly to their possessors in terms of comfort and survival.

Allergic Dogs

After confirming early reports that occasional dogs develop spontaneous pollen allergies resembling human hay fever, asthma, and dermatitis, Patterson, Pruzansky & Chang (56) showed that the responsible antibody was skin-sensitizing for the species, fixed itself persistently to the skin, transferred systemic anaphylaxis to normal dogs, was heat-labile, and nonprecipitating, and that it migrated with the β-globulins—qualities typical also of human reagins. Sparing their few sensitive animals, they (65) immunized normal dogs with ragweed antigen and with such proteins as bovine serum albumin (BSA), procuring nonprecipitating antibodies of γ_1-globulin type and, in occasional dogs, also precipitins that migrated with the γ_2-components. The latter exhibited a sharp, "flocculating" form of precipitin curve with its antigen, conferred the Arthus reaction on the guinea pig, hastened the clearance of circulating antigen, and, in contrast to the results of others (59), failed for some reason to give the PCA response in the guinea pig. The nonprecipitating factor caused no Arthus, postponed the clearance of antigen by precipitins, and gave a negative PCA result in the guinea pig. Neither antibody transferred Prausnitz-Küstner reactivity to normal dogs. Nonprecipitating antibody resembled human blocking factor in its heat stability and competitive qualities.

The dog's elaboration of three types of antibody resembling those of man makes it an ideal model for examining antibody relationships in the serum disease-like disorders. The puzzling failure of dogs with precipitins to evince Arthus responses to their injections might well rest on a blocking role played by coexistent nonprecipitins. The further surprise of encountering anaphylactic episodes early in the injection courses suggests that reagins may have been induced transitorily. In unpublished studies, the writer, attempting to induce blocking antibodies experimentally in a healthy, young man with a highly simplified conalbumin, unexpectedly observed

local urticaria and humoral reagins several days after a booster repository in vegetable oil had been injected under the skin. Prausnitz-Küstner activity rose to a peak on the third day of reagin production and disappeared on the sixth. A sharp rise in blocking antibody titer ensued. The normergic subject returned, when the experiment ended two months later, to his daily consumption of eggs with customary tolerance, despite diminishing persistence of the acquired cutaneous responsiveness. This reaginic phenomenon might explain reports (66) of asymptomatic reagin induction in occasional normergic recipients of experimental pollen emulsion. The evanescent nature of the reagin production we observed robs these much publicized reports of their adverse implications for repository therapy. In any event, the normal dog offers an ideal opportunity to explore just such developments. As will be suggested later, he also provides a setup suited to the study of precipitins for wasp venom, notably their clinical role and the impingement on that role of nonprecipitating antibodies.

Insect Venom Allergies

Although venoms of bees and wasps contain such pharmacologically active substances as histamine, serotonin, and kinin (67), the amounts received in a few stings are too limited to provided primary toxicity for man and large mammals. These haptens may, however, along with the enzymes, hyaluronidase, lecithinase, and cholinesterase, which commonly accompany them (68), underlie hymenoptera anaphylaxis in man. Manifestations of sensitization resemble serum disease at the outset, appearing more rapidly with subsequent exposures until finally response may be immediate and life-threatening. Skin-sensitizing antibodies and blocking antibodies are usually to be found in the bloodstream, and precipitins have been reported in beekeepers (69) as well as in a man who died from wasp stings (70); results being positive by both Ouchterlony (71) and gravimetric precipitin tests. Although 42 allergic sera failed, in the hands of Terr & McLean (72), to form bands with antigen in agar gel, 13 of the 26 sera examined in the guinea pig provided positive PCA responses when challenged with honeybee extract, three when yellow jacket extract was used, and none when polistes or baldfaced hornet body extract comprised the challenge. The degree of success with the PCA test bore no relation to the intensity of intracutaneous reactions. Brown (73) has recently stated that most hymenoptera-sensitive persons exhibit circulating precipitins before, but not after, injections of whole body extract, several untreated patients were likewise losing this Ouchterlony manifestation. Occasional accidental stings evoked no adverse sequelae in them. Although she postulated no mechanism, it seems likely that the acquired tolerance and the undetectability of precipitins both rested on coexistent blocking antibodies which successfully competed against fixed reagins and circulating precipitins. They might also have accounted for the PCA reactions of Terr (72). If the protective hypothesis for blocking antibody is correct, it is reason-

able to infer that not only persons with acute anaphylactic backgrounds, but others with periarteritis referable to venom-precipitins, might benefit from specific immunization where future contacts cannot be avoided. The rationale is dual, as the innocuous blocking factor would neutralize any venom received accidentally, thereby forestalling the risk of anaphylaxis and of Arthus-like responses, while at the same time preventing the booster effect that fresh venom exerts on reagin and precipitin output. These assumptions merit investigation in experimental animals.

Antigens in Hymenoptera Venoms

Extensive explorations have been undertaken by Shulman, Langlois & Arbesman (74) on *Apis mellifera* (honeybee), *Polistes exclamans* (polistes), and *Vespula pennsylvanicus* (yellow jacket). Although zone electrophoresis proved rather undiscriminating, ultracentrifuge revealed five peaks for whole body extracts in contrast to three to four noted in venom released by trituration from its frozen sac. Ouchterlony's techniques later showed (75) venom of the honeybee and yellow jacket to contain two, polistes three, antigens for the rabbit, whereas whole body extracts exhibited four or five, one of these showing a line of identity with a venom antigen. Immunoelectrophoresis analyses indicated far greater complexity, 9 to 13 lines appearing with whole body extracts, whereas one to five were seen in venoms. Polistes appeared to be the most complex, sharing two of its five antigens with the sacless body, compared to one antigen noted in venoms of honeybee and yellow jacket. More recent examination of honeybee venom procured by electrical excitation (76, 77) indicates one prominent and one minor line, the former being absent from the sacless body extract, the latter exhibiting the reaction of identity with a body constituent (78). Prausnitz-Küstner tests with sting-sensitive patients' and with beekeepers' sera revealed a predilection for sacless body specificity in the latter, and for venom specificity in the former, caused no doubt by a difference in the antigens contacted by inhalation and by accidental sting. Their suggestion that body antigens might eventually induce immunity against pure venom seems casuistic, and uneconomic from both the practical and theoretical viewpoints.

After failing during cross-tests with gel diffusion techniques to demonstrate antigens common to honeybee and yellow jacket, they (79) succeeded in detecting a minor one by immunoelectrophoresis. For polistes and yellow jacket, two mutual antigens were revealed, one restricted to their venoms and one to their bodies. Whereas some patients responded, in intracutaneous and Prausnitz-Küstner tests (80), to one species only, there was a general tendency to exhibit species cross-reactions in hemagglutination studies after courses of whole body mixed extract. This probably rested on blocking antibody synthesis for the numerous antigens of the complex mixture. In Prausnitz-Küstner studies which neutralized the serum against one part of the insect, then challenged it with the other, they were unable to procure

any response to venom after the sacless body antigens had been exhausted, and vice versa. Although they attributed this to mutuality of antigens [a concept disproved by their later demonstration of unique activities in venom (78)], it was probably referable to site hyporesponsiveness (32) induced by their use of the *in vivo* type of reagin neutralization, instead of the *in vitro* one with its several necessary controls. As remarked by Pruzansky et al. (81), "reliability of the Prausnitz-Küstner method using intradermal desensitizing injections for the study of allergenic relationship must be questioned. This criticism may not apply to the test tube 'neutralizaion' technic." Use of the *in vitro* technic did not obscure the existence of unique antigens in each of the five hymenoptera venoms we examined (82). Another contribution to the negative cross-test results of Shulman (80) may have been the introduction of three freeze-thaw steps into the extraction process for venom sacs (74).

Brown (83) has recently confirmed, for the intracutaneous use of whole body honeybee extracts, our observation (82) that normal persons regularly respond in a restrained way to test with yellow jacket venoms by the intracutaneous, ophthalmic, and live sting tests.

Clinical inquiry into the role of common antigens in injection therapy has now been made (84). A practical need had been generated by our success with petrolatum: Arlacel A emulsions of polistes venom in nine persons highly susceptible to polistes stings (85). Wishing to extend this single, annual repository method to yellow jacket allergy and lacking live wasps of this type in early spring when such injections must be given, we resorted to the use of the perennially available polistes. With one exception among the 24 patients who received a polistes venom repository and nine others given fluid polistes venom, all 25 members, who submitted to deliberate sting challenge with one to three yellow jackets four to six months later, exhibited tolerance. The exception may represent an unusual orientation toward the unique antigen of yellow jacket venom. The others often exhibited elevated ophthalmic resistance prior to their tolerated stings, thus comprising a new model for demonstrating the correlation between blocking antibody and clinical immunity in an objective manner.

In view of the evidence that antibodies in sting allergies are oriented toward venom rather than the whole body of the insect, it seems unfortunate that extracts of the latter are being so widely used for immunization. The small amount of venom in such extracts is perhaps inadequate for sting-protection, and may explain occasional deaths among those adhering faithfully to the protracted injection schedule. (One was reported in August 1965 in a Wilton, Conn., man under such therapy for two years, the latest treatment having been taken only a week prior to his lethal sting.) Furthermore, the innumerable foreign proteins in commercial preparations, containing as they usually do all the soluble constituents of five species, is a bid to long-term complications like periarteritis (86). Perhaps the introduction of electrically ejaculated venom will overcome industrial

prejudice against a once-yearly treatment that employs simple venom (84). This change would at least exclude the current practice of freezing the stock of insects, with its risk of antigenic damage. (The trituration of frozen venom sacs may, by the way, have encouraged the escape of cell contents from sac walls into venom, thereby contributing body antigens to Shulman's venom preparations and rendering them more complex than ejaculates. In favor of a degree of mutuality between venom and body antigens, however, we have observed that venomless male wasps cause a small amount of whealing when crushed bodies are placed on human scratches, evoking about one tenth the response provided by concurrent test with venom.)

Allergy to Penicillin

This small molecule has come to be the most frequent cause of serum disease and anaphylaxis in man. A skin test before its therapeutic use has, until recently, provided no reliable clue to susceptibility. In seeking a solution, Levine, according to a recent review (87), combined theoretical chemistry with Landsteiner's principles (88), then applied activity tests to animals and man. It was evident that penicillin G lacked the chemical structure to effect covalent linkage with proteins, thereby becoming a complete antigen that could evoke antibody synthesis in the body. Since penicillin-allergic patients were obviously forming antibodies, *in vivo* pathways had to be postulated that would explain the hapten's antigenicity. Intermediate and final forms developed in the laboratory exhibited activity in animals and man. The major antigen was found to be benzylpenicilloyl, a determinant group, after it became conjugated to body proteins. The point of its attachment to the carrier also contributed to the specificity of its antibodies. Benzylpenicilloyl stems from an isomer of penicillin, benzylpenicillenic acid, which forms spontaneously through a simple rearrangement of penicillin G and which is highly reactive with proteins, conjugating with their lysine amino groups. Brandriss (89) has recently presented experimental arguments for another pathway to the benzylpenicilloyl-specificity, involving direct conjugation *in vivo* of the β-lactam carbon of penicillin G to the lysyl amide of protein without participation of benzylpenicillenic acid.

Levine found that haptenic groups other than benzylpenicilloyl were formed to a lesser extent in the body, two of these "minor" determinants being the cysteine-mixed disulfide groups of D-penicillamine and of D-benzylpenamaldic acid. A fourth possible determinant was the benzylpenicillenic acid disulfide group. These conclusions were based on responses given by guinea pigs, with penicillin G induced contact dermatitis, when patch-tested with penicillin G and its degradation products. Rabbits immunized with penicillin G emulsion were observed to acquire abundant antibodies of benzylpenicilloyl specificity according to quantitative precipitin technics, PCA test in the guinea pig, and passive hemagglutina-

tion. Benzylpenicilloyl specificity was confirmed by inhibition studies with a univalent hapten, benzylpenicilloyl-aminocaproate. Although these animals evinced no activity toward minor determinants, DeWeck (90) detected benzylpenicillenic acid specificity in rabbit anti-penicillin G sera.

Man, according to Levine (87), elaborates two antibodies in response to penicillin G, one detected by hemagglutination while the other (reaginic) type is active in intracutaneous and Prausnitz-Küstner tests. Whereas only the benzylpenicilloyl specificity has given results with hemagglutination, three different specificities have been encountered among reagins. One is benzylpenicilloyl oriented; another is reflected in penicillin G tests, as well as tests with benzylpenicilloate and occasionally also with D-penicillamine, indicating specificity for the two minor determinants mentioned above. Still another specificity was encountered in Prausnitz-Küstner studies which revealed positive results with penicillin G but negative ones with benzylpenicilloate, D-penicillamine, benzylpenicilloyl, benzylpenicillenic acid specificities. Indeed, its dedterminant group has not been discovered. A fourth reaginic specificity, for the mixed disulfide group of benzylpenicillenic acid, has been reported by Parker et al. (91) but could not be confirmed (92).

Inasmuch as penicillin gives rise to benzylpenicilloyl-specific hemagglutination in animals and in nearly all persons who receive it, as well as in some who have not, regardless of whether reagin output and clinical episodes are involved, it is simpler for the nonce to assume that these antibodies are induced blocking antibodies without implication for disease. More urgent at the moment is the function of reagins. Budd, Parker & Norden (93), testing 22 persons who had exhibited adverse developments three months earlier, observed intracutaneous responses to benzylpenicilloyl-polylysine in 20 and penicillin G reactions in only three. Among 41 such patients of Levine & Price (92), 29 per cent gave cutaneous wheal-and-flare responses to benzylpenicilloyl-conjugates while only two persons evinced penicillin G and benzylpenicilloate reactions. The latter had experienced symptoms immediately after treatment. It seems likely that the two sets of data would have yielded a higher incidence of test responses to penicillin G had the immediate reactors to treatment been segregated from those with accelerated and serum-like disease manifestations. Among a group of 120 patients with assorted adverse developments to past therapy, Sherman's group (94) noted a downward shift in the incidence of intracutaneous reactivity as time elapsed after the episode. Whereas an intracutaneous response to benzylpenicilloyl-polylysine developed in 67 per cent of those whose interval had been two years or less, the incidence fell to 54 per cent when others with intervals up to three years were included. Only 6 per cent responded to penicillin G test, and marked whealing was restricted to persons with immediate adverse episodes. In another group of 59, who were tested just before penicillin G therapy, only six positive reactions were noted, all to benzylpenicilloyl-

polylysine. None of the six reactors showed untoward sequelae to penicillin G treatment, thereby resembling the 26 benzylpenicilloyl-reactive patients of Rytel (95) who exhibited full tolerance under the same circumstance.

Levine & Fellner (96) observed that all seven of their subjects with past episodes of immediate onset were cutaneously reactive to penicillin G, whereas nine of 14 patients with delayed symptoms responded to intracutaneous test with benzylpenicilloyl-polylysine. In a large random group, the latter conjugate evoked reactions in only 3 per cent. Of 23 reaginic sera examined to date by Siegel (97, 98), for patients with immediate adverse developments to penicillin G, 21 of the Prausnitz-Küstner responses were oriented toward penicillin G and penicilloate, reflecting activity for minor determinants.

Although the penicillin G-detected reagin, according to the foregoing early studies, begins to emerge as the mediator and forewarner of anaphylaxis in man, this step ahead in our knowledge opens a floodgate to other questions. Has the benzylpenicilloyl reagin no untoward role? Or is that role being masked clinically by the ubiquitous hemagglutination antibodies formed toward this determinant, the blocking powers being locally overridden in Prausnitz-Küstner and intracutaneous tests that perchance employ excessive strengths? Are there no hemagglutination antibodies for minor determinants, or could they be detected by conjugates of the penamaldic acid and penicillamine haptens? To what are serum disease symptoms referable—benzylpenicilloyl reagins, or undetected precipitins? Could the latter be demonstrated by tests with the several determinants if those haptens were first attached to established, or as yet undiscovered, body carriers? Such questions should ignite a smouldering interest in many a young scientist and bring, hopefully, tomorrow's answers.

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CONTENTS

SIDELIGHTS OF AMERICAN PHARMACOLOGY, *Carl A. Dragstedt* . . . 1
AZTEC PHARMACOLOGY, *E. C. del Pozo* 9
RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY, *Alfred Burger and Anilkumar P. Parulkar* 19
CARDIOVASCULAR PHARMACOLOGY, *Francis J. Haddy and Jerry B. Scott* 49
ELECTROLYTE AND MINERAL METABOLISM, *L. G. Welt, J. R. Sachs, and H. J. Gitelman* 77
THROMBOLYTIC AGENTS, *Anthony P. Fletcher and Sol Sherry* . . . 89
AUTONOMIC NERVOUS SYSTEM: NEWER MECHANISMS OF ADRENERGIC BLOCKADE, *E. Muscholl* 107
EFFECT OF DRUGS ON SMOOTH MUSCLE, *G. Burnstock and M. E. Holman* 129
NONSTEROID ANTI-INFLAMMATORY AGENTS, *Charles A. Winter* . . 157
COMPARATIVE PHARMACOLOGY, *William G. Van der Kloot* 175
PERINATAL PHARMACOLOGY, *Alan K. Done* 189
ANTIBACTERIAL CHEMOTHERAPY, *I. M. Rollo* 209
ANTIVIRAL CHEMOTHERAPY, *Hans J. Eggers and Igor Tamm* . . . 231
DRUGS AND ATHEROSCLEROSIS, *Karoly G. Pinter and Theodore B. Van Itallie* 251
RENAL PHARMACOLOGY, *John E. Baer and Karl H. Beyer* 261
TOXICOLOGY, *L. I. Medved and Ju. S. Kagan* 293
ANTIBODIES OF ATOPY AND SERUM DISEASE IN MAN, *Mary Hewitt Loveless* 309
DRUGS AND RESPIRATION, *Christian J. Lambertsen* 327
ANESTHESIA, *Leroy D. Vandam* 379
ON THE MODE OF ACTION OF LOCAL ANESTHETICS, *J. M. Ritchie and Paul Greengard* 405
REVIEW OF REVIEWS, *Chauncey D. Leake* 431
INDEXES 445
AUTHOR INDEX 445
SUBJECT INDEX 471
CUMULATIVE INDEX OF CONTRIBUTING AUTHORS, VOLUMES 2 TO 6 . 492
CUMULATIVE INDEX OF CHAPTER TITLES, VOLUMES 2 TO 6 . . . 493