ANNUAL REVIEWS Further
Quick links to online content

Copyright 1973. All rights reserved

ALLERGY TO PENICILLIN AND RELATED ANTIBIOTICS: ANTIGENIC AND IMMUNOCHEMICAL MECHANISM

6568

G. T. STEWART

Department of Epidemiology and Preventive Medicine, University of Glasgow, Ruchill Hospital, Glasgow, Scotland

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

Hypersensitivity to penicillins is of considerable practical and theoretical significance in medicine and biology. Its practical significance in medicine is familiar, in so far as it causes alarming adverse reactions and limits the use of this group of antibiotics; its theoretical significance is that it reveals how a range of relatively simple, nontoxic molecules can be converted into extremely powerful immunizing and sensitizing antigens.

Implicit in these facts are biological anomalies that emphasize the essential differences between allergy and toxicity, on the one hand, and between immunity and allergy, on the other. The molecules of penicillin and of its commonly used derivatives are nontoxic in comparison with most other antibiotics and with most therapeutic substances. Penicillin itself (benzyl-penicillin, penicillin G) is remarkably nontoxic insofar as it is well tolerated for months, parenterally, in daily doses of 100 mg/kg and for years at lower doses even when there is severe impairment to renal excretion (1). When toxicity does occur, it is more likely to be due to electrolyte imbalance from the potassium or sodium cation of the salt than from the penicillin molecule itself (2, 3). The same lack of toxicity is seen with the main derivatives of penicillin: phenoxymethyl penicillin (penicillin V) can be given for years with safety in daily doses of 10–20 mg/kg to children with rheumatic heart disease; α-amino penicillin (ampicillin) is well tolerated parenterally in daily doses of 400 mg/kg in patients with gram-negative osteomyelitis or endocarditis; dimethoxy penicillin (methicillin) has often been used in similar doses to treat patients with staphylococcal osteomyelitis or pyemia, and the carboxypenicillin to treat infections due to *Ps. pyocyanea*. Other new penicillins, such as oxacillin, cloxacillin, dicloxacillin, nafcillin, and hetacillin, all show similar if slightly less nontoxicity. Yet all are capable of eliciting allergic responses in sensitized subjects and all are liable in varying degree to be cross-allergenic (4).

Against this tolerance of penicillins by the majority of persons must be set the extreme intolerance of a minority who may experience life-threatening reactions from a single therapeutic dose or even a skin-test dose. Thus, at one extreme, we find a seriously ill patient with diminished renal function tolerating 10 mega-units of benzyl penicillin (5.5 g) at a single injection, while another subject, in good health, reacts violently to a millionth part of that dose, i.e., to 10 units

(5.5×10^{-6} g). Between these limits one finds many persons who react with varying severity to doses at any level, though in general it may be said that allergic responses are not often observed after high doses (10–100 mg/kg or more) of the commonly used penicillins.

It is a paradox that the least toxic antibiotic molecules are the most highly allergenic. But most, if not all, persons receiving penicillins develop some antibodies to penicillin conjugates, so intolerance is not caused by antigenicity per se. The question, therefore, arises as to whether the minority of persons who are intolerant because of hypersensitivity are identical with the minority who are hereditarily destined to become atopic, or whether there is another explanation. Upon the answer to this question depend a number of subsidiary questions about how to avoid or control adverse reactions to the penicillins.

INCIDENCE

For various reasons, it is impossible to obtain a true figure for the incidence of hypersensitivity to penicillins. Numerous estimates have been made but they all vary from one another according to where the subjects are seen, how they are questioned, whether diagnosis is by report or observation, whether they are challenged with penicillin, and so on. The fact is that all reports and many observations cannot escape inherent fallacies. Patients' recollections are often unreliable; observers seldom quote control series; skin tests and other diagnostic methods are never 100% accurate; hypersensitivity comes and goes; patients with a convincing history of an observed reaction often tolerate penicillin while others with negative histories have reactions; and so forth: the literature is so full of contradictions, anomalies, and inconsistencies that review is difficult and conclusion impossible.

Reviewing the literature in 1965 (2) and again in 1970 (5), the writer and his colleagues suggested that, in the absence of high risk factors such as topical application or over-the-counter sales, the general incidence in Western European and North American populations lay between 1% and 10%. Nothing has been read since then to contradict this estimate which may, therefore, suffice for the present review, which is concerned with mechanism of allergy. But it is very relevant to mechanism to emphasize the importance of risk factors. Levine and his colleagues (6) detected antipenicillin antibodies in the sera of 303 patients aged 22–80 irrespectively of any history of therapy with penicillins, the main difference between individuals being that atopic patients responded 3–4 times as often as nonatopic patients by forming skin-sensitizing antibodies. In a hospital survey, Cluff and his colleagues (7) found that 32 out of 408 (7.8%) of patients receiving penicillin showed signs of allergy but only 7 of these 32 gave positive skin reactions to penicilloyl polylysine. Dawson & Segal (8) found evidence of penicillin-induced hemolytic anemia in 8.8% of 132 patients receiving high doses of penicillin, whereas van Dellen et al (9) testing 240 patients with histories of reactions found that 57 had positive skin tests to either benzylpenicillin, methicillin, or ampicillin but only 5 to all three. Green and his colleagues (10) got a history of allergy from 71 (18%) of 400 patients treated with penicillin for endo-

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org
by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

carditis; 22 (39%) of 56 experienced further reactions when given more penicillin as compared with only 5% of those who had no history of allergy.

Ampicillin carries a much higher risk of skin eruptions than other penicillins, though not all of these rashes are allergic. An indirect estimate based on drug rashes reported in 12,638 patients who received ampicillin gave an incidence of 2.8% (11) but estimates made from direct surveillance are usually higher, ranging from about 4% to 40% (12–15). Shapiro et al (16) quoted the risk rate attributable to the drug as 7.7%, and later raised their estimate to 9%. Patients with mononucleosis or leukemia are especially liable to develop maculo-papular or urticarial eruptions if treated with ampicillin (see below).

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

FORMATION OF THE ANTIGEN

According to the classical theory of antigenicity propounded by Landsteiner (17), a small molecule cannot be antigenic unless it unites as a hapten with a larger molecular that serves as a carrier. If the carrier molecule is not recognized as foreign or unwanted by the recipient, the determinant of antigenic specificity is the hapten. Landsteiner showed that nonantigenic native proteins were converted into powerful, highly specific antigens, by coupling with reactive molecules like dinitrophenols. The keystone of Landsteiner's theory, which has found wide acceptance among immunologists, is that the coupling bond is covalent, i.e., one involving a sharing of electrons by adjacent atoms. Formation of a stable covalent bond depends upon the presence of a free reactive group in the haptenic molecule. Penicillin salts, as normally used, contain no such free group and form unstable, reversible, noncovalent bonds with protein (18). From this arose the idea in Eisen's laboratory in St. Louis that the effective hapten was one of the reactive degradation products that formed readily from penicillin in aqueous solution (19, 20). The degradation products originally identified were D-benzylpenicillenic acid, D-benzylpenicillamine and D-α-benzylpenicilloic acid, of which the penicillenic acid derivative readily formed a covalent bond with protein to give a potent multivalent antigen (21, 22). In addition to inducing specific antibodies in rabbits and guinea pigs, these penicillenate and penicilloyl conjugates also elicited wheal- and flare-responses in persons known to be hypersensitive to penicillin. They were, therefore, useful as skin-test reagents but were likely to sensitize nonsensitive individuals. To avoid this risk the St. Louis workers prepared penicilloyl conjugates with a simple polymer (penicilloyl polylysine, PPL) which elicited skin-test responses in hypersensitive subjects without stimulating the production of reaginic antibodies. The specificity of this reagent was shown by inhibition of the skin-test response by the corresponding univalent haptens, N-penicilloyl-epsilon-aminocaproic acid and its aldehyde, which are structurally similar to the penicilloyl lysyl groups on proteins or on polylysine carriers.

Although penicillins seem to induce an antibody response in most recipients, a specific antibody response cannot be produced experimentally unless the antigen is multivalent, i.e., has several haptens attached to the protein or peptide carrier. Theoretically, a bivalent antigen should be potent but, as Parker has pointed out

(22), small bivalent molecules are much less effective than larger carriers even when the ratios of penicilloyl groups (0.0005–0.05 mμEq) are equal, presumably because of steric hindrance to the attachment of two antibody molecules to two closely placed haptenic groups.

In addition to penicilloyl groups, it became apparent from further work by Parker, Levine, de Weck and their colleagues that other degradation products could act as haptens with varying frequency and efficiency. Penicillenates have already been discussed but their importance was to some extent discounted by the observations (4, 23) that the nucleus of the penicillin molecule, 6-aminopenicillanic acid (6-APA) was antigenic and that this substance and substituted derivatives such as the phenoxymethyl and phenoxyethyl penicillins and dimethoxypenicillin (methicillin), which cannot form penicillenates, were capable of eliciting allergic responses. The other haptenic determinants include penicilloates, penicillamine, penaldate, and penicoyl conjugates (24). To exclude all possibilities, it is necessary to test hypersensitive subjects with a combination of conjugates as well as penicillin itself and unconjugated products which can presumably form conjugates in skin tests by linking with tissue proteins. Levine (25) has characterized these substances collectively as minor determinants and has identified obscure cases of hypersensitivity by using a "minor determinant mixture".

Up to this point, it was assumed that conjugation occurred in vivo because of covalent bonding of penicillin derivatives with tissue proteins. However, Feinberg (26) and Stewart (27) had meanwhile begun a series of experiments which eventually showed that penicillins could form allergenic macromolecules in other ways. Feinberg (28) has now reported earlier experiments in which he and J. H. C. Nayler made penicillin into a mixed anhydride treatment with ethychloroformate and then reacted this with protein to link the intact penicillin via its carboxyl group with a free amino group on the protein. Rabbits immunized with this complex yielded antibodies, presumed to be specific for the intact penicillin molecule, which did not cross-react with a penicilloyl determinant. A bis-penicillin (p-phenylene-bis-penicillin) was then prepared and conjugated with protein at one end, leaving intact penicillin at the other. This antigen cross-reacted with both antipenicillin and antipenicilloyl sera. Feinberg also viewed with suspicion the finding, reported above, the 6-APA had immunogenic properties and found that he could separate from crude 6-APA by dialysis an immunogenic protein with penicilloyl specificity. Stewart (27, 29) found that 6-APA, freed from the contaminating protein, lost its ability to evoke a wheal- and flare-response in hypersensitive human subjects. This protein, which had already been identified as a stationary ninhydrin-positive spot in ascending chromatograms of 6-APA, cross-reacted with antipenicilloyl antibodies. When fractionated, it showed low electrophoretic mobility, appeared to be of high molecular weight and was partly denatured. A similar proteinaceous residue was then obtained from standard (B.P. and U.S.P.) preparations of benzyl penicillin. This also had penicilloyl specificity and was intensely reactive in skin tests, causing in one sensitized subject an alarming anaphylactic response in a scratch test. Removal of this protein by various techniques (dialysis, gel, or membrane filtration) yielded

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org
by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

a "pure" penicillin that was tolerated in skin test doses by some highly allergic subjects who reacted briskly to the parent antibiotic sample. Three volunteers, known to be hypersensitive to penicillin, tolerated therapeutic doses of batches from which the macromolecular proteinaceous residue had been removed but reacted to small skin test doses (10 μg) of the parent batches of standard B.P. and U.S.P. penicillin.

Experimental studies in rabbits and guinea pigs (29–31) and later studies in primates, showed that natural penicillins and cephalosporins, and some of their semi-synthetic derivatives, regularly contained macromolecules capable of inducing and eliciting antibody responses with penicilloyl or cephalosporoyl specificities, and limited cross-reactivity. In general, three components could be separated by UV column chromatography.

A. A component of high molecular weight (>5000) with $K_{av} \rightarrow 0$, little or no antibacterial activity, and spectroscopic profiles in the UV, IR, and NMR which were entirely different from the overall spectra of starting material and from the other fractions.

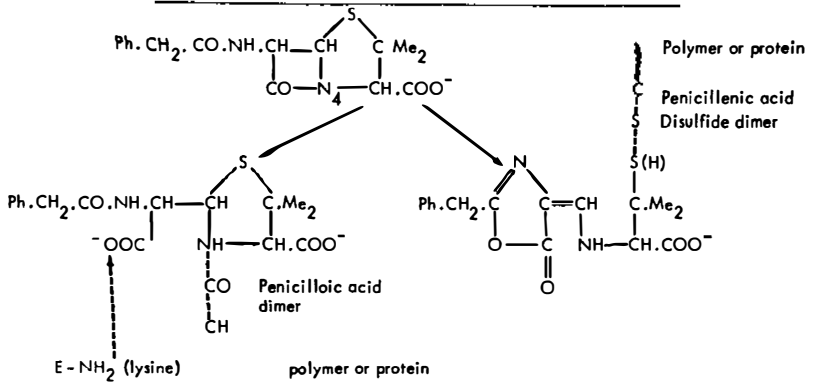
B. Residues of intermediate molecular weight (1000–5000) with K_{av} 0.4–0.6, lowered antibacterial activity and some resemblance in spectrometric profiles to the parent antibiotic or its degradation products.

C. Low molecular weight fractions (<1000) with $K_{av} \rightarrow 1$, high antibacterial activity (90–100% of expected value) and preservation of the overall spectrometric profile of the parent antibiotic.

This was the general pattern but individual antibiotics yielded fractions that differed considerably from each other and have been described in detail elsewhere (31). Benzylpenicillin, obtained from various well-known manufacturers between 1964 and 1967 and tested during that time, always yielded a high molecular weight ($>10{,}000$) residue in amounts varying from the lower limit of detection (10 mg/100 g) to 48 mg/100 g, the usual range being 17–30 mg. This residue when dried was a fluffy, nondialyzable, creamy powder, moderately soluble in water. It gave reactions for protein, sometimes for carbohydrate, and yielded on acid hydrolysis a quantity of ammonia and, in lesser quantity, a range of amino acids including asparagine, alanine, glycine, leucine, lysine, methionin, and threonine. Valine and dimethylcysteine or related products were always present, and these with glycine were the predominant amino acids produced when dialyzed or ultrafiltered penicillin was hydrolyzed. Some unidentified ninhydrin-positive material plus degradation products of penicillin were also present in the residue. IR spectrometry showed total absence of the stretching vibrations of the lactam ring of penicillin at 1760 cm^{-1} but marked absorption of UVL at 322 $m\mu$, indicative of penicillenic acid. This fraction, therefore, had the properties of a protein complex, presumably of mycelial origin, penicilloylated or otherwise conjugated to penicillin or to its degradation products or to both.

Intermediate fractions from benzyl-penicillin (mol wt 1000–5000, average approximately 2000) gave no reactions for protein and yielded only glycine, valine, and dimethylcysteine in much smaller quantities, with degradation

DIMERIZATION AND CONJUGATION OF BENZYLPENICILLIN



products, on acid hydrolysis. Free sulfur or sulfhydryl groups were present, and the absorbance at 322 mμ in UV of benzylpenicillenic acid. Lyophilization yielded a hard, insoluble plastic material. Nuclear magnetic resonance spectrometry showed preservation of the 5 phenyl and 6 dimethyl protons of benzylpenicillin, but loss of lactam structure (Figure 1). This fraction, therefore, had the properties of an inactive polymer, shown later to be formed by sub-units of degraded penicillin.

The low molecular weight (>1000) fractions from benzylpenicillin had all the properties, including antibacterial activity, of the parent antibiotic. In unbuffered aqueous solution, it degraded like the parent antibiotic to benzylpenicillenate and then slowly polymerized to give insoluble material similar to the intermediate fraction. The rate of degradation was minimized by buffering at pH 7.0 and by storage at 4°C in the dark.

Independently of these results, the presence of protein residues in benzylpenicillin was reported by Batchelor et al (26) and by Pederson-Bjergaard (32). Macromolecular residues, not necessarily proteinaceous, were also reported in semisynthetic penicillins and in cephalosporins (31). Knudsen et al (33) and Robinson (34) confirmed Stewart's finding that removal of the proteinaceous residue from the antibiotic removed also the power to elicit wheal- and flare-reactions in some hypersensitive subjects. De Weck, Schneider & Gutersohn (35) subsequently confirmed the presence of penicilloyl macromolecules but were uncertain of their relationship to allergy.

In tests performed with batches of standard (USP-BP) penicillins obtained from various manufacturers after May 1967 (the date of publication of first reports about the allergenic residues), proteinaceous residues were invariably low (<11 mg/100 g) or undetectable though polymer, formed as before by condensation of degradation products, was regularly detected. This was confirmed by Dursch (36) and de Weck et al (35). It seemed likely, therefore, that prior to May 1967 there were present, in representative batches of penicillin prepared by reputable manufacturers, small quantities of penicillinoylated proteinaceous

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org
by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org
by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

residues that could induce and elicit allergic responses even in minute doses (37).

Preparations of 6-APA, cephalosporin C, and 7-aminocephalosporanic acid, as used in manufacturing processes, also yielded equivalent or greater amounts of proteinaceous residues after fractionation (50–400 mg per 100 g) but showed a lesser tendency than benzylpenicillin to polymerize (31). These residues, like the residues from pre-1967 benzylpenicillin, were complexes of various amino acids with degradation products of the antibiotics and sometimes traces of carbohydrate and unidentifiable organic material. The semi-synthetic derivatives of these natural products, however, seldom contained detectable protein though without exception they polymerized in solution to yield macromolecules of varying complexity, which have been described elsewhere (38). The macromolecular complex from ampicillin is of special interest in view of the high incidence of rashes noted in some patients and some conditions after therapy with this drug (12–16). Ampicillin, with a free basic group on the α-carbon, is more reactive than benzylpenicillin and may not need to degrade to form dimers that form the structural nuclei of small polymers (mol wt 1400–5000) constituted by carboxyl- or carbonyl-amino linkages. NMR studies show preservation of the phenyl and probably of the thiazolidine rings; SH-groups, free sulfur, and the penicillenic acid homolog are not present; acid hydrolysis yields only phenylglycine or phenylalanine, valine and cysteine derivatives, which are the amino acid residues expected from a condensation polymer. Though substantially free from intrinsic protein, possibly due to refinements in the extraction procedure, this polymer readily conjugates with added protein and can, therefore, form an immunogenic complex with penicilloyl specificity. It may be noted that ampicillin is usually cross-allergenic with benzylpenicillin in hypersensitive subjects and it has been claimed (39, 40) that removal of the macromolecular complex reduces its allergenicity.

The nature of the proteinaceous residues in the natural antibiotics has not been finally settled. The most likely source is the mycelium of the crude brew and this may account for most of the protein detected in benzylpenicillin, which owes its allergenicity, specificity, and extraordinary potency to penicilloylation. In 6-APA, however, it seems likely (26, 28) that some of the protein is enzymatic, derived from the amidase added to the fermentation brew to remove the side chain of the precursor, which is usually penicillin G or V.

The antigens formed by natural penicillins and their semi-synthetic derivatives, and by the related β-lactam antibiotics, the cephalosporins, are, therefore, variable in structure, in origin, on conjugation patterns, and in re-activity. They can work together to produce complex hypersensitivity or singly to produce highly specific sensitivity in which only one antigenic determinant is identifiable —though this is not usual. With penicillins, though not often with cephalosporins, clinical cross-reactivity is the rule, hence a patient sensitized to one form of penicillin is liable to react with varying severity to other penicillin antigens. Sometimes an antigen can immunize but not elicit a reaction (41), while other antigens, such as the penicilloyl polylysines, can readily elicit specific responses without immunizing experimental animals or test subjects. Experimental work

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

indicates that immunogenicity requires a macromolecular conjugate and that the major determinant in such conjugates is a penicilloyl (41–43) or cephalosporoyl (44, 45) hapten, usually but not always formed by a degradation product of the antibiotic attaching itself as a monomer, dimer, or small polymer to two or more sites of the protein, or protein-carbohydrate carrier. The site of penicilloylation on the carrier may be a peptide chain with an N-terminal cysteine liberated by the action of penicilloic acid. A similar reaction occurring on the peptide polymer of the antibiotic can readily lead to stable bonding with protein and to the formation of complex antigens. If penicillenic acid is involved, the complex can contain four diastereomeric penicilloyl groups (41) which further complicate the problem of identifying the main haptenic determinants and may explain why attempts to block reactions with hapten inhibitors have not always been successful. The variety of degradation products, dimers, and polymer sequences now known make it extremely unlikely that any one monomeric hapten is a dominant determinant. It may be questioned also if irreversible, covalent bonding is really necessary to confer hapten-specificity on a carrier: benzylpenicillin and its main derivatives unite very readily with proteins, without detectable degradation, to give conjugates with penicilloyl specificity; the dimers and polymers can obviously form cross-links, hydrogen bonds, and other aggregates. It seems more likely that various aggregating mechanisms are operative and that complex antigens are formed by hydrophilic as well as functional groups, by aminolysis and by van der Waals forces between molecules that are known, in other situations, to be the main cohesive agencies in highly-ordered biomolecules (46). This question is of more than theoretical significance because the widespread use of PPL for skin tests is based on the assumption that a simple polymer is not antigenic and is safe as an elicitor; whereas in fact it can cause severe reactions (47) and might enter an antigenic complex as readily as some other penicillin polymers.

Whereas the immunizing antigen must be large, the elicitor of an allergic response can be large or small. Most of the macromolecular complexes described above can elicit reactions but so also can small molecules such as unconjugated benzylpenicillin, a bis-penicilloylated diamidobutane, and penicilloates that can conjugate by nonpenicilloyl routes such as a penicilloate-cystine disulfide interchange (41). If conjugates are used as elicitors, two or more determinants per carrier molecule are necessary—in contrast to immunizing conjugates that are effective with one haptenic determinant per molecule. Whether small molecules elicit by rapid conjugation with tissue carriers or as dimers or small polymers is therefore uncertain, but it is certain that, in some allergic subjects, pre-formed conjugation is not necessary for eliciting a response, though it is impossible to exclude dimerization in penicillin solutions.

The first stage in formation of a dimer from benzylpenicillin can result from degradation of the penicillin to either penicilloic acid or penicillenic acid. Penicilloic acid is liberated by any one of several mechanisms favoring β-lactam hydrolysis. The consequent fall in pH then permits further degradation to penicillenic acid to form a dimer (Figure 1) which is the essential subunit of a small polymer (38). Either the dimer or the polymer can act as a hapten and

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org
by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

bridging agent on a protein carrier with penicilloyl, penicilloate, and penicillenate specificities. Alternatively, benzylpenicillin can degrade to penicillenic acid, which can acylate with the N4 of the thiazolidine ring (see Figure). Either or both routes can, therefore, lead to small peptide polymers that have limited potency as immunizing agents but can act as elicitors and (if conjugated by covalent bonding or aminolysis to protein) as antigens and elicitors, which are probably as potent as any in any immunochemical system. From the practical viewpoint it should be noted that these antigens and elicitors can be effectively eliminated from therapeutic preparations of penicillin by appropriate extraction and filtrations, or by using chemically-pure 6-APA as a starter. Dimerization cannot be entirely prevented but the formation of the precursors is minimized by buffering to pH 7.0 and it is no longer defensible to prepare unbuffered penicillin for therapeutic use.

Ampicillin polymerizes more readily but is much less likely to form mixed dimers. The essential subunit is probably a dimer of ampicilloic acid, but, with this formed, a variety of other possibilities exist for subsidiary polymerization via the α-NH_2 and the carboxyl (50). The polymer thus formed has a molecular weight of about 3500 and, though by itself weakly immunogenic, readily forms much larger complexes with protein to yield highly immunogenic agents with somewhat narrower specificity than those derived from benzylpenicilloic but with a considerable potential for provoking allergy. Collaborative studies between hospitals have shown considerable variation in the occurrence of cutaneous eruptions and between the various commercially-available brands of ampicillin (51). Removal of macromolecular complexes (39, 40) from therapeutic preparations brings a striking reduction in the incidence of skin rashes.

CLINICAL REACTIONS

The outstanding clinical feature of hypersensitivity to penicillins is its unpredictability. It can occur, in severe form, in patients who have no history of any other allergy, in persons who have previously received penicillin for days, months, or years with impunity, even in persons who have never received penicillin therapeutically (2, 52). Having occurred once, it may recur and intensify for years, or it may become quiescent. It may appear often at the onset of therapy or during therapy. It may appear, recede, then reappear. It is not dose-related: large doses may be well tolerated while small doses, even skin test doses of a few micrograms, can cause severe local or general reactions. Indeed, there is some evidence (53, 54) that a single large dose may overcome hypersensitivity, and it is seldom that a severe reaction follows a very large dose administered parenterally.

On clinical rather than immunological grounds, reactions can be classified as follows:

A. *Immediate*: A local or general reaction, including anaphylaxis, occurring within a few minutes of giving a dose, and seldom after 20 minutes. The first symptom is often pruritis in the naso-labial region or at the site of a skin test. If sensitization has resulted from topical application (which is still practiced in some countries), the pruritis may be felt in the original site of

sensitization after the antibiotic is given orally or parenterally. The usual cutaneous manifestation is urticaria. If mucous membranes are affected, the result may be asthma, rhinitis, or laryngeal oedema. This may be followed by cardiovascular collapse. But there may be no warning symptoms and anaphylaxis may occur immediately, with dyspnea and hypotension. Unless there is prompt intervention with oxygen and epinephrine, anaphylaxis or laryngeal oedema may prove fatal.

B. *Accelerated* reactions occur about 2–48 hours after a dose, usually in the form of urticaria, though other skin rashes, fever, abdominal crises, nephropathies, and laryngeal oedema may occur.

C. *Late reactions* are those occurring after a delay of 3 or more days, sometimes after the antibiotic is stopped. Symptoms and signs are protean: serum sickness with arthralgia, often persistent; urticaria and eczemas; purpura, with or without thrombocytopenia; lupus erythematosus; anemia with or without hemolysis. Pathological changes include Stevens-Johnson syndrome; pulmonary and hepatic cellular infiltrates; nephropathies, and myocarditis.

The common features in these reactions are sensitization at one or more sites by cell-bound antibody, and release of histamine, kinins, and other products (55). There are some reactions, however, that cannot be explained by this conventional pattern of allergic response. The best known, and perhaps the most common, is penicillin-induced hemolytic anemia (56, 57). This occurs in patients who have received large doses of benzylpenicillin intravenously but are not, or not necessarily, hypersensitive in terms of the responses described above. The direct Coombs test is positive, the indirect test negative with normal erythrocytes, but positive when the erythrocytes are exposed to penicillin. Hemagglutinating IgG antibodies are present and the penicillinized red cells have a shortened half-life. The syndrome abates when penicillin is stopped, recurs when it is restarted. The antibodies are not usually specific for the penicilloyl hapten, so it is possible that other antigenic determinants are at work (58).

Penicillin-induced hemolytic anemia is obviously an unusual immunologic disorder, provoked perhaps by the effect of large doses on the red cell. There are several other rare but unexplained syndromes associated with penicillins, notable among which are the oliguria-hematuria syndrome produced mainly by methicillin (59) and the skin eruptions caused by ampicillin. The oliguria-hematuria-neutropenia syndrome of methicillin and the isoxazolyl penicillins (oxacillin *et seq.*) does not necessarily invoke an immunological mechanism and will not be discussed further here. But ampicillin deserves special attention because it provokes a very curious reaction, which may offer a clue to the nature of abnormal antibody responses.

From the start of its therapeutic career (60) ampicillin has been foremost of the penicillins—and perhaps of any drug—in causing skin eruptions, some of which were urticarial, some maculopapular, with a total incidence ranging from 3–11% of patients treated. A mean figure of attributable risk recently reported by Shapiro et al from an extensive survey was 9% (51) and it is true to say

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

that eruptions are so common, especially in children, as to be expected everywhere by physicians using ampicillin (60–63). There has always been doubt about the relationship of most of these eruptions to allergy and it now appears, from the work of van Arsdel and his colleagues (64), that only the urticarial eruptions, amounting to one-third of total eruptions, can be ascribed to allergy. The reason for the other maculo-papular eruptions remains obscure.

The most curious side-effect of ampicillin, first noted by Patel (65) and by Pullen et al (66) is in patients with mononucleosis (67, 68), 80–90% of whom develop skin eruptions. Some of these eruptions are urticarial, some erythematous, some macular or maculopapular, often with intense pruritis. Other signs of hypersensitivity, such as oedema and arthralgia, are the exception rather than the rule and antihistamines are of little benefit. Antipenicillin antibodies may be present. The relationship, if any, to the hetero-typical hemagglutinating antibodies of mononucleosis is not established. Recently it has been found that patients with leukemia may also have an abnormally high incidence of skin eruptions if they receive ampicillin. In addition, it appears from further indirect observations by Shapiro et al (69) from the Boston Collaborative Drug Surveillance that concomitant administration of allopurinol raises the incidence of ampicillin rashes from 7.5–22.4% or, alternatively, that ampicillin raises the "rash rate" of patients receiving allopurinol from 2.17–22.4%. The clue here may be hyperuricemia in patients receiving allopurinol, for there is independent evidence (70) that ampicillin is more likely to cause rashes in patients with hyperuricemia, irrespectively of the action of other drugs. This may be the link with leukemia, in which blood uric acid levels are often high.

There are, therefore, three additional syndromes associated with therapy with penicillins, which have features in common with immediate, accelerated, or delayed hypersensitivity reactions, but are not wholly explained by an allergic pattern of response or by disorder of the immunologic mechanism. It is important, therefore, to examine the immunologic mechanisms in some detail to see where the line can be drawn between allergy, as represented by urticaria and anaphylaxis, and idiosyncrasy, as represented by the rashes occurring in mononucleosis and the oliguria-hematuria syndrome.

IMMUNOLOGY

Various antibodies are concerned in these different reactions (71). The immediate reactions are usually considered to be mediated by IgE (72) though other immunoglobulins IgG and IgM are usually present. The IgE may be specific for any or all of the various antigenic determinants and is the antibody associated with the wheal- and flare-responses in positive skin tests.

The role of IgG and IgM is much less certain. Many persons who tolerate penicillin have these antibodies in the blood, sometimes in quite high titer. As tested by hemagglutination, these antibodies tend to be specific for benzylpenicilloyl-α-diastereoisomeric conjugates in human subjects and in experimentally immunized animals (73). This is shown by inhibition by univalent

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

benzylpenicilloyl amines (74) and, in the minority of cases where inhibition does not take place, other antigenic determinants may be involved (75). This is a conspicuous feature of penicillin-induced anemia (58).

Irrespectively of the type of the reaction, patients with a history of allergy are more likely to have high titers of these antibodies than control subjects, especially when tests are performed soon after their reactions—a period during which skin tests may be negative. According to Levine and his colleagues (76), higher titers are obtained if hemagglutination is performed in dextran media. By this means, these workers claim that there is a serological difference between cases with accelerated or late urticarial reactions, who have high IgG titers, and those with morbilliform rashes who have high IgM titers. Patients giving positive reactions to skin tests with penicilloyl polylysine have high IgG antibodies, which diminish in time (77). In many cases of accelerated and late reactions, both reactions may be obtained initially but then one or other may rise or fall. This probably depends upon how much antibiotic has been given. Continued administration increases the titer of circulating IgG, which may then act as a blocking antibody and inhibit combination of the penicillin antigen with skin- or organ-sensitizing antibody according to whether the IgG and IgE have identical specificities. If it is remembered that IgG is usually penicilloyl-specific whereas IgE may have affinity for a wider range of determinants, the numerous exceptions and anomalies are easier to explain, though not to understand.

Most patients who have had immediate reactions have given positive skin reactions to benzylpenicillin and/or penicilloyl-polylysine, but only about half have measurable titers of IgG (76). A high-titer penicilloyl antibody is suggestive of allergy, but the presence of such antibody does not prove allergy nor does absence of antibody exclude it. Skin-tests behave similarly: an immediate reaction to one or more penicillin antigens is suggestive but not conclusive nor is absence exclusive, especially since the skin test is liable to become negative immediately after a severe reaction to a dose. For diagnostic purposes, the only reliable exception is the occurrence of severe urticaria and systemic signs after a skin test, which is a sure but dangerous pointer to severe hypersensitivity.

Various other tests have been used as diagnostic and research tools: basophil degranulation, passive transference of antibodies to guinea-pigs, primates, and humans, lymphocyte transformation, assays of histamine, radio-isotopic labelling of antigens and antibodies. As in many other branches of medical science, the advance has been in technology rather than in prevention or cure, for these new methods, elegant as they are, have not as yet revealed anything important about the antigen, the antibody, or the disorder that was not already known. It is, therefore, important to attempt to make some order out of the present confusion if only to arrive at a hypothesis upon which to base further action in a field as difficult scientifically as it is dangerous clinically. The only useful practical point emerging out of many years of painstaking research is the fact that some reactions are averted by removing preformed macromolecular complexes from the antibiotic. But a great deal of knowledge is now available about the mechanism of the reaction and about the inherent difficulties in diagnosis and management.

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org
by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org
by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

CONCLUSIONS

At the risk of oversimplification, the essential results of about 30 years of research upon the side effects of penicillin may be reduced to these essential findings.

A. Penicillins are immunogenic, as demonstrated by their ability to produce various specific antibodies in blood and tissues of patients and experimental animals.

B. The immunogenic property depends upon the ability of penicillin, its degradation products and polymers, and related substances to form highly specific haptens by formation of macromolecules, before or after administration.

C. The immunogenic potential is invariable but allergy is relatively uncommon, variable in time, duration, and pattern of occurrence, and may be problematic mainly in atopic subjects.

D. Allergy manifests itself mainly by direct, easily recognized reactions but can also produce indirect effects, which appear to result from immunological disorder or interactions with disturbed metabolic processes.

It should be remembered also that penicillins are peptides, formed biosynthetically by a fusion of amino acids (2) and that the cyclic fusion of these peptides, natural to moulds, are foreign to man, especially when administered parenterally. Yet man does encounter penicillin-like molecules naturally, because they are produced by household moulds and by dermatophytes such as *Trichophyton mentagrophytes* (78). Therefore he is exposed, and liable to be sensitized, by handling mouldy produce or by cutaneous mycosis without ever receiving penicillin. If he then receives penicillin as an injection he is receiving a concentrated booster dose of antigenic substance. He develops antibodies, which increase with subsequent injections, especially if preformed antigenic macromolecules are present in the injection material. This process results in the high titers of IgG and IgM that usually result from parenteral treatment but it does not explain hypersensitivity or idiosyncrasy, for most persons tolerate penicillins very well indeed.

To try to explain this, we borrow first the axiom of allergy that a proportion of persons are atopic and liable to be sensitized by antigens that are neutralized or rejected painlessly by nonatopic individuals. It seems reasonable to suppose that some of the people who become allergic to penicillins are in this atopic minority, who manufacture an excess of IgE or fail to produce enough neutralizing antibody. Even in those, it is fair to assume that IgG and IgM can act as blocking antibodies, diverting the antigen from the sensitizing antibody, and that the excess of monomeric antibiotic itself may act as a monovalent inhibitor of union between antigen and antibody. Also, an injection induces stress, and stress liberates epinephrine, which is itself an inhibitor of histaminic-mediated allergic responses. Hence, if these immunologic and physiological mechanisms are operating normally, the injection (or oral dose) is tolerated. But imbalance at any point—excess IgE, lack of blocking antibody, a more potent antigen, failure of

physiological response—is liable to lead to allergy. In some people, susceptibility is heightened by atopy, by topical exposure, by injection of preformed conjugates, by receiving a preparation full of degradation products due to faulty manufacture or storage. In such persons, a small dose may trigger a violent mobilization of sensitizing antibody and anaphylaxis, though a large dose may inhibit the reaction by swamping all antibody receptors.

The principle of invariance and individuality of protein in mammals (79) imposes a condition, now confirmed by experiment, that antibodies are mass-produced rather than tailor-made, and that the body's information system recognizes not just the noxious hapten but the stereochemical complex of antigen plus preformed antibody. If this concept is accepted, the search for every possible antigenic determinant becomes endless and futile: any hapten or combination of haptens can be antigenic but will be neutralized if antibody synthesis and mobilization are efficient, as in the majority of persons receiving antibiotics. Intolerance arises when the mechanisms of stereospecific recognition and neutralization are excessively stimulated or are themselves imperfect or under strain. If the antibiotic contains preformed macromolecules as degradation products, it is liable *ipso facto* to initiate or elicit allergy and, in this sense, allergy is preventable by the enforcement of stricter standards of purification by manufacturers and regulating authorities. But if antigenic complexes are formed by purified antibiotic in vivo, there is as yet no certain way of avoiding allergy except by withholding the antibiotic. This is a sad conclusion, since penicillins are difficult if not impossible to replace in the treatment of some major infectious diseases.

TABLE 1. Tests for Allergy to Penicillins and Related Antibiotics

Test	Antibody	Specificity
Intradermal tests with benzylpenicillin, derivatives and PPL	Cell-fixed IgE	Penicilloyl, penicillenate. Dimeric and polymeric Conjugates. Minor determinants. Ampicillin dimers.
Passive cutaneous anaphylaxis	Circulating IgE	Same Cephalosporoyl.
Hemagglutination	IgG and IgM	Penicilloyl, cephalosporoyl
Direct Coombs	Immunoglobulins	Nil
Indirect Coombs	Immunoglobulins	Uncertain
Basophil degranulation	Cellular	?Penicilloyl
Lymphocyte transformation	Cellular	Penicilloyl, cephalosporoyl
Histamine-release from tissue	Cellular	Uncertain
Radio-isotope	IgG	Penicilloyl
Bacteriophage reduction	IgG, IgM, IgE	Penicilloyl

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.

LITERATURE CITED

1. Bigby, M. A. M., Jones, F. A. 1949. *J. Obst. Gynaec.* 56:636–47
2. Stewart, G. T. 1965. *The Penicillin Group of Drugs*, Elsevier, New York
3. Tullett, G. L. 1970. *Wisc. Med. J.* 69:216–17
4. Stewart, G. T. 1962. *Lancet*, 1:509
5. Stewart, G. T., McGovern, J. P. 1970. *Penicillin Allergy: Clinical and Immunologic Aspects*, Thomas, Springfield, Ill.
6. Levine, B. B., Redmond, A. P., Fellner, M. J., Voss, H. E., Levytska, V., 1966. *J. Clin. Invest.* 45:1895–1906
7. Smith, J. W., Johnson, J. E., Cluff, L. E. 1966. *New Eng. J. Med.* 274:998–1002
8. Dawson, R. B., Segal, B. L. 1966. *Arch. Int. Med.* 118:575–79
9. Van Dellen, R. G., Walsh, W. E., Peters, C. A. 1971. *J. Allergy*, 47:230–36
10. Green, G. R., Peters, G. A., Geraci, J. E. 1967. *Ann. Int. Med.* 67:235–40
11. Knudsen, E. T. 1969. *Brit. Med. J.* 1:846–47
12. *Brit. Med. J.* 1972. 1:195–96
13. Steiniger, U. 1969. *Med. Klin.* 63:146–47
14. Phillips, W. E. 1971. *J.A.M.A.* 217:913–15
15. *Lancet*, 1969. 1:993
16. Shapiro, S., Slone, D., Siskind, V. 1969. *Lancet*, 2:969–72
17. Landsteiner, K. 1962. *The Specificity of Serological Reactions*, Dover Publ., New York
18. Clarke, H. T., Johnson, J. R., Robinson, R. (Eds.) 1949. *The Chemistry of Penicillin*, University Press, Princeton, N.J.
19. Levine, B. B. 1960. *J. Exp. Med.* 112:1131–56
20. De Weck, A. L., Eisen, H. N. 1960. *J. Exp. Med.* 112:1227–47
21. Parker, C. W., de Weck, A. L., Kern, M., Eisen, H. N. 1962. *J. Exp. Med.* 115:803–19
22. Parker, C. W., Shapiro, J., Kern, M., Eisen, H. N. 1962. *J. Exp. Med.* 115:821–38
23. Chisholm, D. R., English, A. R., McLean, N. A. 1961. *J. Allergy* 32:333–42
24. Budd, M. A., Parker, C. W., Norden, C. W. 1964. *J.A.M.A.* 190:203
25. Levine, B. B., Redmond, A. P., Voss, H. E., Zolov, D. M. 1967. *Ann. N.Y. Acad. Sci.* 145:207
26. Batchelor, F. R., Dewdney, J. M., Feinberg, J. G., Weston, R. D. 1967. *Lancet* 1:1175–77
27. Stewart, G. T. 1967. *Lancet* 1:1177–83
28. Feinberg, J. G. 1970. In *Penicillin Allergy*, eds. Stewart, G. T., McGovern, J. P., 90–105. Thomas, Springfield, Ill.
29. Stewart, G. T. 1967. *Antimicrob. Ag. Chemother.* 7:543–49
30. Butcher, B. T., Stewart, G. T. 1968. *Antimicrol. Agents Chemother.* 8:515–18
31. Butcher, B. T., Stewart, G. T. 1970. In *Penicillin Allergy*, eds. Stewart, G. T., McGovern, J. P. 111–32. Thomas, Springfield, Ill.
32. Pederson-Bjergaard 1969. *Acta Allerg.* 24:303–25
33. Knudsen, E. T., Robinson, O. P. W., Croydon, E. A. P., Tees, E. C. 1967. *Lancet*, 1:1184–88
34. Robinson, O. P. W. 1968. *Antimicrob. Ag. Chemother.* 8:550–52
35. De Weck, A. L., Schneider, C. H., Gutersohn, J. 1968. *Int. Arch. Allerg.*, 33:535–67
36. Dursch, F. 1968. *Lancet*, 1:1005
37. Stewart, G. T. 1968. *Lancet*, 1:1088
38. Stewart, G. T., Stanfield, M. K., Butcher, B. T., Waegle, S. 1970. Biopolymerization of Peptide Antibiotics in *Liquid Crystals and Ordered Fluids*. eds. Johnson, J. F., Porter, R. S. Plenum Press, New York
39. Knudsen, E. T., Dewdney, J. M., Trafford, J. A. 1970. *Brit. Med. J.* 1:469–71
40. Croydon, E. A. P. Personal communication
41. Schneider, C. H. 1970. In *Penicillin Allergy*, eds. Stewart, G. T., McGovern, J. P., 23–51. Thomas, Springfield, Ill.
42. Levine, B. B. 1965. *Fed. Proc.* 24:45–56
43. De Weck, A. L., Blum, G. 1965. *Int. Arch. Allergy* 27:221–30
44. Batchelor, F. R., Dewdney, J. M., Gazzard, D. 1965. *Nature* 206:362–64
45. Schneider, C. H., de Weck, A. L., 1965. *Nature* 208:57–59
46. Stewart, G. T. 1969. *Mol. Cryst. Liquid Cryst.* 7:75–90
47. Ettinger, E., Kaye, D. 1964. *New Eng. J. Med.* 271:1105–08
48. Resnik, S. S., Shelley, W. B. 1966. *J.A.M.A.* 196:740–41
49. Levine, B. B. 1965. *J. Immunol.* 94:111–17

50. Stanfield, M. K., Butcher, B. T., Stewart, G. T., 16 Sept. 1971. Communication to *American Chemical Society*, Washington, D.C.
51. Arndt, K. A., Parrish, J., with Boston University Medical Center. 1972. *Ampicillin Rashes: A Report.* Boston Collaborative Drug Surveillance Program, Harvard Univ. Med. Sch.
52. Calvert, R. J., Smith, E. 1955. *Brit. Med. J.* 2:302–04
53. Katsilabros, L. 1967. *Rev. Med. Moyen Orient* 24:346–48
54. Metz, G., Pevny, I., Metz, J. *München Med. Wochenschr.* 29:1341–48
55. Perelmutter, L., Eisen, A. H. 1970. *Int. Arch. Allergy* 38:104–12
56. Strumia, P. V., Raymond, F. D. 1962. *Arch. Int. Med.* 109:603–10
57. Beardwell, C. G. 1964. *Proc. Roy. Soc. Med.* 57:332–34
58. Petz, L. D., Fudenberg, H. J. 1966. *New Eng. J. Med.* 274:171–73
59. McElfresh, A. E., Huang, N. 1962. *New Eng. J. Med.* 266:246–48
60. Stewart, G. T., Coles, H. M. T., Nixon, H. H., Holt, R. J. 1961. *Brit. Med. J.* 2:200–03
61. Crow, K. D. 1970. *Trans. St. John's Hosp. Dermatol. Soc.* 56:35–36
62. Speck, W. T. 1971. *Clin. Pediatr.* 10:59–60
63. Kronig, B., Dennig, H. 1970. *Arzneim Forsch.* 20:1930–34
64. Bierman, C. W., Pierson, W. E., Zeitz, S. J., Hoffman, L. S., van Arsdel, P. P. 1972. *J.A.M.A.* 220:1098–1100
65. Patel, B. M. 1967. *Pediatrics* 40:910–11
66. Pullen, H., Wright, N., Murdoch, J. McC. 1967. *Lancet*, 2:1176–77
67. Coskey, R. J., Paul, L. 1969. *Arch. Dermatol.* (*Chicago*) 100:717–19
68. Weary, P. E., Cole, J. W., Hickman, L. M. 1970. *Arch. Dermatol.* (*Chicago*), 101:86–91
69. Boston Collaborative Drug Surveillance Program. 1972. *New Eng. J. Med.*, 286:505–07
70. Jaffe, I. A. 1970. *Lancet* 1:245–46
71. Levine, B. B. 1966. *New Eng. J. Med.* 275:1115–25
72. Ishizaka, K., Ishizaka, T. 1968. *J. Allergy* 42:330–63
73. Levine, B. B., Fellner, M. J., Levytska, V. 1966. *J. Immunol.* 96:707–16
74. Van Arsdel, P. P. 1970. In *Penicillin Allergy*, eds. Stewart, G. T., McGovern, J. P., 59–77. Thomas, Springfield, Ill.
75. Thiel, J. A., Mitchell, S., Parker, C. W. 1964. *J. Allergy* 35:399–407
76. Levine, B. B., Redmond, A. P., Fellner, M. J., Voss, H. E., Levytska, V. 1966. *J. Clin. Invest.* 45:1895–98
77. De Weck, A. L. 1964. *Nature* 202:975–76
78. Smith, J. M. P., Marples, M. J. 1964. *Nature* 201:844
79. Monod, J. 1971. *Chance and Necessity.* Collins, London

Annu. Rev. Pharmacol. 1973.13:309-324. Downloaded from www.annualreviews.org by RENSSELAER POLYTECHNIC INSTITUTE on 12/15/11. For personal use only.