

Penicillin Allergy – Can the Incidence be Reduced?

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Introduction

The various penicillins prepared from *Penicilium* species by fermentation (benzyl penicillin and phenoxymethyl penicillin) or by enzymatic or chemical modification of the benzyl penicillin nucleus (the semisynthetic penicillins) are among the most widely used and valuable antibiotics currently available. However, since the introduction of the drug for treatment of bacterial infection in April 1942, a drawback in penicillin therapy has been the occurrence of adverse reactions. In the early days of the penicillin era, when amorphous preparations were used, impurities derived from the fermentation process were blamed for these adverse reactions (33). With the advent of crystalline penicillin preparations, antigens formed *in vivo* during therapy by chemical reactions between penicillin or its degradation products and substances in the body were believed to be the culprits (27).

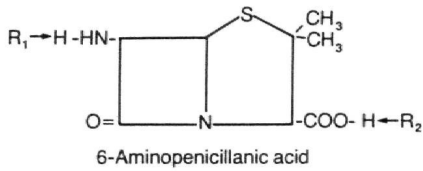
A number of comprehensive reviews on various aspects of penicillin allergy have been written in recent years (2, 6, 24, 25, 26, 28, 60, 61, 72). In the present review I will therefore concentrate on certain aspects of antigen formation and on reactions required for the manifestation of penicillin allergy. Some ideas are brought forward as to what can be done to

decrease the incidence of severe allergic reactions to penicillin.

Clinical manifestations

It is important to emphasize that truly allergic reactions are only a part of the adverse reactions seen during penicillin therapy. The time of onset and location of allergic reactions to penicillins vary. All *allergic* reactions require a phase of sensitization to penicillin antigen, inducing immunocompetent cells to proliferate and differentiate and to produce antibodies and lymphokines. After the sensitization period re-exposure to penicillin antigen may elicit an allergic inflammation. The antigen responsible for the allergic inflammatory reaction is not necessarily the same as the antigen which originally sensitized the patient. Thus, penicillin-derived structures formed by different penicillins (Fig. 1) show gross cross-reactivity. Furthermore, requirements considering epitopes and carrier on the antigen sensitizing to penicillin and eliciting a penicillin allergic response differ considerably (2, 6).

The allergic reactions to penicillins include immediate reactions as well as allergic reactions with later onset. Anaphylaxis, appearing within 1 h after exposure to penicillin, represents the most severe type of allergy to penicillin. Hypo-



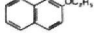
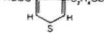
R ₁	R ₂	Generic Name
	H	Benzylpenicillin (Penicillin G)
	H	Phenoxymethylpenicillin (Penicillin V)
	H	Phenethicillin
	H	Ampicillin
	H	Amoxicillin
	-CH ₂ OCOC(CH ₃) ₃	Pivampicillin
	H	Cloxacillin
	H	Dicloxacillin
	H	Flucloxacillin
	H	Methicillin
	H	Carbenicillin
	H	Azidocillin
	$\begin{array}{c} CH_3 \\ \\ -CH-OCO-C_2H_5 \end{array}$	Bacampicillin
	H	Nafcillin
	H	Ticarcillin

Fig. 1. The basic structure of the penicillin molecule, 6-aminopenicillanic acid, and of the groups attached, to give some of the common penicillins.

tension and death may occur without any other symptoms. Pruritus, urticaria and angioedema,

laryngeal oedema and, more rarely, rhinitis and asthma, may also appear. One to 72 h after exposure of a sensitized individual to penicillin antigen accelerated allergic reactions can sometimes be seen. These reactions may manifest themselves as urticaria, angioedema, and occasionally pharyngeal oedema.

Late adverse reactions are sometimes seen more than 3 days after penicillin administration. Such reactions include skin eruptions such as exanthemas on the trunk and proximal extremities. Morbilliform exanthemas seen during therapy have been claimed to be mediated by immunity to penicillin (26). Erythematous reactions may also appear, which may rarely develop into Stevens-Johnson's syndrome. Other late reactions with doubtful causal connection to penicillin antigens are serum-sickness-like reactions, possibly related to the *Herxheimer* reaction, which is attributed to flooding of the body with large quantities of dead microorganisms and microbial products as a result of the antimicrobial therapy (29). Such effects might result in reactions like urticaria and arthralgia persisting for some time. Skin rashes seen in connection with ampicillin therapy are often associated with certain abnormalities due to the disease (*vide infra*).

Sometimes vasculitis and haemorrhagic disorders might develop (9, 20, 23, 59, 70). When high dose therapy is used, thrombocytopenia or generalized purpura, haemolytic anaemia and leukopenia are seen rarely. Occasionally, nephropathy (14, 20, 21, 60, 61, 72) with a basophil infiltrate (20) is seen. Whether this is of penicillin allergic etiology is uncertain, however.

Incidence of penicillin allergic reactions

There are a number of factors influencing the frequency of penicillin allergy, including route of administration. For example, topical application is efficient in sensitizing the patient, therefore penicillin is not used therapeutically on the skin. Injections of penicillin as a depot preparation, as in procain penicillin, also have a high incidence of sensitization (Table 1). The lowest incidence of penicillin allergic reactions is seen

Table 1

Incidence of adverse reactions to penicillin therapy

Penicillin	Incidence of adverse reactions %	Reference
<i>Parenteral</i>		
Penicillin G	2.5	39
Ampicillin	7.2*	39
Procain penicillin	5	79
Benzathin penicillin	0.3	79
<i>Oral</i>		
Fenoxymethylpenicillin	0.3	79
Penicillin G	1.4	39
Ampicillin	3.5*	39

* Significant difference $P = 0.002$.

after oral therapy (12, 24, 39, 79) and to my knowledge only six fatal cases have been reported in the literature after oral therapy. As has been discussed by de Swarte (24), intermittent administration of the drug is associated with a higher risk for sensitization of the patient than a prolonged administration of penicillin in high doses.

Age seems to influence the incidence of allergic reactions to drugs including those to penicillin. Thus, children and older people show considerably less drug allergic reactions than young adults (24, 61). In adults, atopic individuals seem to have a higher incidence of both penicillin allergy (12, 24) and pseudo-allergic reactions to the compound (12).

The reported figures of the frequency of penicillin adverse reactions in exposed adults vary from less than 1% to about 10%. For the most frequently used drugs in Sweden 1980–1981 (74) there were 82 reported* cases of adverse reactions against phenoxymethyl penicillin per 30.7 DDD $\times$ 10 and 70 against amoxycillin per 4.1 DDD $\times$ 10. The incidence of reported allergic reactions to penicillin in Sweden 1965–1982 is shown in Table 2 (74).

The frequency of anaphylactic-type reactions was estimated in 1968 by Idsøe et al. (40) at about 0.004–0.015% of patients undergoing parenteral therapy. Whether these figures are valid today is not known. A higher incidence has been reported for unspecific skin reactions. From figures presented by de Weck (26) (Table 3) it seems that the incidence of penicillin allergy, particularly of the anaphylactic type, has decreased, whilst generalized exanthemas show a tendency to increase.

Between 1965 and 1982 there were 17 reported cases of anaphylactic reactions to penicillin tablets in Sweden (74).

Immunogenicity of penicillin

The penicillin conjugated antigens belong to three categories. High molecular weight proteinaceous products originating from the fermentation of the benzylpenicillin or phenoxymethyl penicillin are present in many penicillin preparations from most manufacturers (Fig. 2) (3). Despite their usually low amounts, such contaminants can sensitize animals after parenteral administration (Figs. 3a, b) (5, 8, 49). It is possible to decrease the content of such high molecular weight impurities considerably during the manufacturing process, possibly even to a clinically insignificant level.

When infants and children were treated for one period with intravenous infusions of penicillin preparations containing as little as about 1.5 μ g contaminant per gram penicillin substance, they did not form any antibodies to penicilloyl detectable with the enzyme-linked immunosorbent assay, ELISA (5). Furthermore, when adult patients with chronic bronchitis were given repeated intramuscular ampicillin, more penicilloyl specific antibodies did not seem to be induced by the preparations containing small amounts of high molecular weight contaminants compared with the very pure preparations (Fig. 4) (4). On the other hand, administration to mice and rabbits of similar amounts of penicillin containing fermentation products, or experimentally added penicilloylated proteins, clearly induced the formation of antibodies. Studies of patients with histories of

* Report to the Swedish Adverse Drug Reactions Advisory Committee, Uppsala, Sweden, is compulsory.

Table 2

Reported adverse reactions connected with penicillin treatment in Sweden 1980–1982. From Strandberg (74)

Anaphylactic reactions	Penicillin tablets	17
Stevens-Johnson's syndrome	Ampicillin	9
	Phenoxymethyl penicillin	6
Urticaria + oedema	Phenoxymethyl penicillin	67
	Ampicillin	53
	Amoxycillin	31
	Pivampicillin	25
Exanthemas	Ampicillin	262
	Pivampicillin	111
	Amoxycillin	104
	Other penicillins	85

Table 3

Reported cases of penicillin allergy in Switzerland during two periods. de Weck (26)

Main symptom	Period	
	1962–1966 (311 cases)	1971–1976 (231 cases)
Urticaria	201 (33)*	90 (39)
Anaphylactic shock	59 (19)	8 (3)
anaphylactoid symptoms		
Serum-sickness-like disease	43 (14)	6 (3)
Angioneurotic oedema	28 (9)	31 (14)
Generalized exanthema	25 (8)	65 (28)
Contact dermatitis	15 (5)	3 (1)
Blood dyscrasia	13 (4)	4 (2)
Generalized pruritus	6 (2)	7 (3)
Local reactions	7 (2)	4 (2)
Asthma	4 (1)	0 (–)
Miscellaneous	9 (3)	6 (3)

* Percentages are given in brackets.

penicillin allergy have shown significantly elevated antibody titers in at most 30% of cases (22, 36). A similar incidence of antibody formation has been found in normal individuals (12). The presence of penicilloyl specific antibodies in up to 97% of randomly tested individuals, irrespective of history of penicillin allergy, reported by Levine et al. (52), cannot be explained unless the exposure to penicillin due to penicillin contamination of, e.g., foods, has de-

Table 4

Ampicillin rash

Frequency of skin rashes is increased when ampicillin is given to patients with:

chronic lymphatic leukemia
infectious mononucleosis
cytomegalo virus infection
virus infections in the respiratory tract
salmonellosis
renal disease
hyperuricemia
allopurinol treatment
Females have higher incidence of reactions than males

creased, and the quality of the penicillin preparations used for therapy has improved.

Experiments during recent years have shown that degradation products with likely sensitizing capacity are formed in the presence of carbohydrate (16, 17, 50, 59), lactate and metal ions (51), all frequent additives to penicillin infusion solutions and peroral penicillin mixtures. An infusion solution with benzylpenicillin and glucose contains degradation products of the penicillin after storage for 16 h (57). Such preparations have a much more pronounced sensitizing capacity than a freshly prepared infusion solution. Patients treated with stored penicillin therefore produce significantly higher antibody titers and lymphoproliferative responses to penicillin antigen than patients treated with freshly prepared penicillin infusion solution (Fig. 5) (57). Similarly, storage of penicillin mixtures for oral administration results in antigen formation (1, 69).

Antibodies to penicilloyl determinants formed from different penicillins cross-react extensively (2, 28, 41, 68). Cross-reactions also occur with antigenic determinants derived from cephalosporins (64) although some of the cephalosporin-derived determinants seem highly specific (42). The cross-reactivity of antibodies raised against various penicillin derivatives may be related to the nature of the antibody response. Thus, some years ago, Atsumi et al. (13) showed in rabbits immunized with

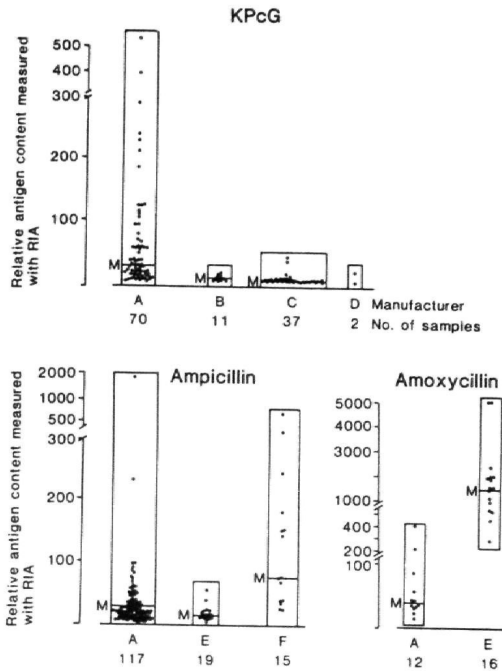


Fig 2. The contents of high molecular weight penicilloylated material measured with a radioimmunoassay (RIA). The numbers of brands are indicated (2–117) of benzylpenicillin (KPcG), ampicillin and amoxycillin from various manufacturers (A–F). From (3).

penicilloyl that a more pronounced specificity of the antibodies developed to the penicilloyl moiety of a certain penicillin than that of another penicillin, early in the antibody response. Later during the immune response, the antibodies showed wider cross-reactivity and reacted with the penicilloyl moieties of various penicillins. The corresponding observation in patients seems to be that antibodies induced by treatment with phenoxymethyl penicillin have higher specificity to the homologous penicillin than to benzyl penicillin, expressed both in skin tests and serological assays like RAST (44). Animal studies have suggested that the degree of this specificity may be related to the nature of the penicillin used for sensitization (7, 48). Thus, antigen conjugates carrying azidocillin were slightly more immunogenic and bound antibodies better than ampicillin and benzylpenicillin. Further studies, using other penicil-

lins with bulky side-chains, are needed to clarify the degree of such specificity.

The IgE antibodies induced by penicillin may mediate immediate allergic reactions to the penicillin-derived antigens. Less is known about the type of symptoms that may result from the development of IgG and IgM antibodies. The serum-sickness-like disease sometimes occurring after penicillin administration is difficult to understand in view of the small amounts of penicillin-derived antigens and specific antibodies expected (2, 6). The reactions could be a consequence of penicillin killing the bacteria, resulting in release of toxic products. These products then cause symptoms in concert with specific antibodies to the bacterial antigens. If penicillin is conjugated on cell surfaces, e.g. after high dose therapy, the antibodies to penicillin may also cause disorders like thrombocytopenia or leukocytopenia directly. Immune complexes

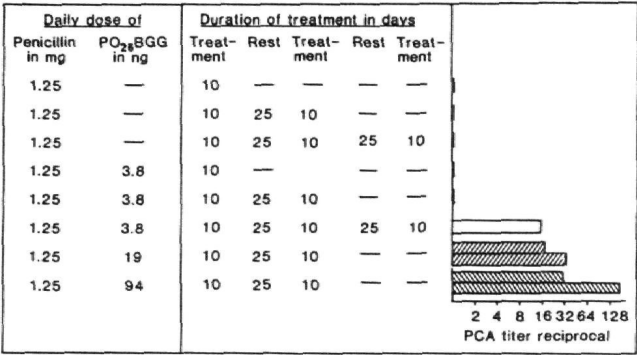


Fig. 3a. The IgE antibody responses in mice measured as heterologous passive cutaneous anaphylaxis (PCA) after 10-day periods of subcutaneous injections of 1.25 mg benzylpenicillin alone or with 3.8–9.4 ng penicilloylated bovine gammaglobulin (PO₂₆-BGG). From (49).

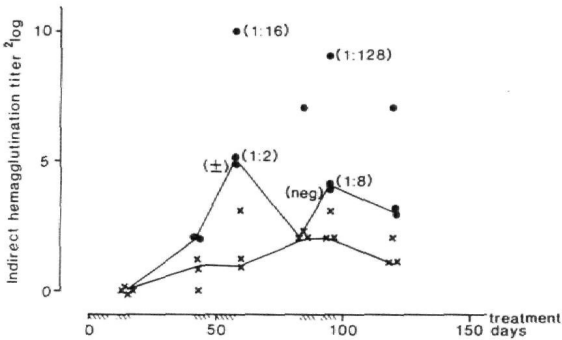


Fig. 3b. The indirect haemagglutination titer ²log in rabbits subcutaneously injected in 10-day periods with 10 µg (●) or 1 µg (×) of PO₂₆-BGG. The figures in brackets indicate the IgE responses measured as homologous PCA. From (5).

may be another explanation for these immune-mediated disorders. For example, antibodies complexed with the drugs quinine and quinidine have a particular affinity to platelets (19). Induction of cell-mediated immunity should be expected if the penicillin conjugates efficiently to body protein (18). Some studies have shown positive lymphocyte proliferation in individuals sensitive to penicillin (34, 35, 65, 76, 77), whereas others have not (32). A cell-mediated immunity may explain the positive epicutaneous test described in patients with penicillin-induced immediate hypersensitivity, as well as some morbilliform exanthemas (26). Due to the

suggested antigen formation *in vivo* during therapy, virtually *all* individuals treated with penicillin should be expected to be immunized and to show positive lymphoblast transformation test results. The present experience is that this is not the case but, that mostly only patients showing allergic symptoms are positive in these tests.

Penicillin antigenic determinants

To understand why penicillin allergy might develop during treatment, and to be able to decrease the incidence of this drug allergy, it is

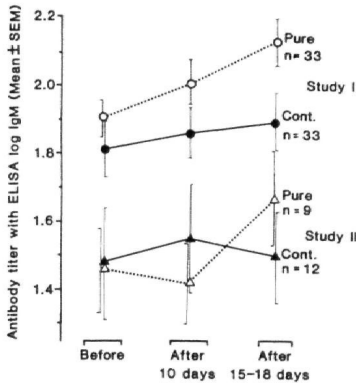


Fig. 4. The IgM antibody responses measured with the ELISA in serum from patients with chronic bronchitis treated for acute infection episodes with intramuscular ampicillin containing about 1.5 μ g (cont.) or less than 0.5 μ g (pure) of high molecular weight contaminating material. From (4).

important to consider how the penicillin-derived immunogens are formed. The penicillin molecule is a structure with capacity to form antigenic determinants on proteins and carbohydrates by chemical reaction with various chemical groups, in particular ϵ -amino groups, COOH and SH groups. The rate of penicillin antigen formation depends on the pH and on the local concentration of penicillin and its metabolites and carrier macromolecules. The chemistry of these formations has been thoroughly reviewed in recent years (2, 45, 66) so only a few major points will be discussed here.

The penicilloyl determinant has been established as the major antigenic determinant in penicillin allergy (2, 6, 26, 28, 60, 61). It is formed directly from penicillins at alkaline or neutral pH. The chemical reaction leading to its formation is catalyzed by imidazole structures (15). In alkaline solutions of penicillin in glucose and sucrose penicilloyl also forms as an intermediate structure, the end product comprising penicilloic acid (16).

The penicilloyl determinant also appears as a result of reaction of penicillic acid (2, 45, 66), but then the penicilloyl occurs as isomers, which makes a different antibody specificity possible. Penicillic acid is formed in minute

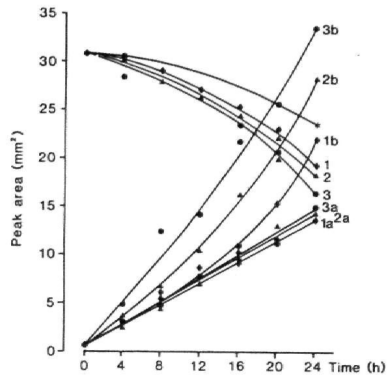


Fig. 5a. The formation of degradation products from benzylpenicillin-glucose infusion solution during storage. 1, 2, 3 indicates different experiments at room temperature. a, b indicates degradation products. ----- indicates benzylpenicillin stored at +4°C.

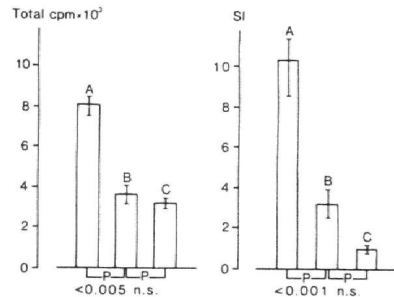


Fig. 5b. The antibody levels in serum and lymphocyte transformation of peripheral blood lymphocytes from (A) patients treated with stored benzylpenicillin-glucose infusion solution containing degradation products according to a); (B) patients treated with freshly prepared infusion solution and; (C) untreated control individuals. From (57).

amounts during manufacture of penicillin and probably also during storage of penicillin, particularly in solution. Due to presence of minute amounts of water also in penicillin powder and tablets, such formation cannot be excluded during storage of penicillin in such forms also, but this is expected to occur to a much lesser extent. The resulting determinants after reactions of the penicillin molecule are shown in Fig. 6. The extent of conjugation of determinants, besides the penicilloyl, to high molecular weight carriers

bodies may be directed to the penicillin (49), and consequently give rise to drug specific inflammation. Virus infections may also enhance the inflammatory responses to penicillin and other substances, e.g. by modulating the activity of the regulator cells or by effects on the adrenergic receptors. These aspects were reviewed recently (2, 6), and are not included here.

Cells conjugated with penicillin may be active in T-cell regulation of the immune system, as was recently shown experimentally. In mice, trinitrophenyl-modified B-cells stimulated T-cells to enhance non-antigen-specific help and suppression of an *in vitro* antibody response. The characteristics of the effects noted were similar to allogeneic effects (30). Such effects can conceivably occur in penicillin-treated patients, inducing inflammatory reactions difficult to diagnose.

Changes of the cell surface may result in toxic effects such as elimination of certain cells or cell functions. This would be expected particularly after high-dose therapy for long periods of time, and there has been a report of two cases of penicillin-induced leukopenia, indicating a direct effect of the penicillin on the maturation of granulocytes in the bone marrow (22). In another report (58), high-dose therapy with benzylpenicillin was shown to induce neutropenia in eight patients. The disorder seemed partly to be of true immunohaemolytic nature and thus due to penicillin-specific antibodies. In addition, in four of the cases, an auto-immune phenomenon could have been implicated, since there were also antibodies directed against the neutrophils.

The adverse reaction commonly called "ampicillin rash" is seen rather frequently during penicillin therapy. Such reactions are most common in connection with particular diseases (Table 4). The mechanism for such reactions is obscure, although cell-mediated immunity to penicillin has been blamed (56, 78). However, if this was the only explanation, all penicillins would induce the same number of reactions, which is clearly not the case; the reactions are most often seen after administration of aminopenicillins. Furthermore, the reactions would

be expected every time the patient gets penicillin treatment, which is not the case either (2, 28, 29, 67). Mitogenicity or polyclonal antibody formation after treatment with ampicillin does not seem to explain these reactions (1, 2), although some studies have indicated this possibility (56, 78). The most probable explanation for these skin reactions appears to be that the amino group on the aminopenicillins causes non-specific effects on leukocyte or lymphocyte function, particularly if this is altered due to other diseases, e.g. during EB-virus infection or chronic lymphatic leukemia. Nearly 100% of ampicillin-treated patients develop skin rashes in connection with these diseases (18, 63). This may relate to the recently indicated higher skin irritating effect of ampicillin compared with that of some other penicillins (48).

Diagnosis of penicillin allergy

The Penicillin Study Group of the American Academy of Allergy has reported a 6-fold higher incidence of penicillin allergic reactions in patients with a history of penicillin allergy compared with patients without such history (37). Diagnosis of possible penicillin allergy in patients can be performed by skin testing, determination of IgE antibodies in serum, and possibly by analysis of lymphocyte reactivity to penicillin antigens, if the latter tests are not performed too early after the allergic reaction (34). Only 3% of patients with negative skin tests are claimed to show penicillin allergic reactions when treated. In contrast, 67–73% of patients with positive skin tests show such allergic responses (37, 75). The incidence of positive tests varies between studies. By using different test reagents, skin tests may provide information about the formation of penicillin antigen and the mechanisms responsible for the reactions to these drugs. Patients who have reacted to penicillin but who show negative skin tests, however, are at risk of reacting again if retreated. This was recently reported by Atkinson & Wheeler (12). In their study, patients with histories of urticaria or anaphylactic symptoms reacted with a probably IgE-mediated reaction on retreatment significantly more often (6.5%) than

patients with other histories of penicillin reactions (0.8%), or without such histories (0.4%).

During recent years a number of reports about skin testing in the diagnosis of penicillin allergy have been published. Two recent articles (71, 73) both describe the results obtained during nearly a decade, and in each study over 700 patients were included. In the first study Sullivan et al. (73) tested 740 patients between 1970 and 1979, with remarkable results. Ninety-five per cent of the patients had experienced a probable allergic reaction to penicillin. The patients were tested with penicilloyl-polylysine, benzylpenicillin, benzylpenicilloic acid, ampicillin and cephalotin. As many as 63% of the patients exhibited positive skin tests after intradermal testing. Somewhat lower figures were recorded after percutaneous testing. The figures were lower still in patients who had had the reaction more than 1 year before. Most of the test reactions were clearly positive or negative when enough test material was used, but 9% of the skin tests were not possible to interpret. The two patients with anaphylaxis showed positive skin tests as did most serum-sickness-like disease. Half of the patients with a history of pruritic macular rash or urticaria during treatment showed positive reactions during testing. It might have been expected, however, that patients with a history of anaphylaxis and urticaria should show positive skin tests more often than patients with exanthemas.

Adult patients younger than 30 years of age had positive reactions more often than older patients (73). Two thirds of the children below 10 years of age showed positive skin tests. To get a truly positive or negative skin test result, benzylpenicillin, benzylpenicilloylpolylysine and benzylpenicilloic acid were needed. The addition of cephalotin and carbenicillin added some information. In view of these data and in view of the specificity of the antibody response it would be wise to include the penicillins with which the patient has been treated.

The high frequency of positive skin test results in the above study is uncommon. Other authors have different experience, particularly in children. Graff-Lonnevig (36) performed an

analysis of 237 children with suspected penicillin allergy and a history of urticaria and exanthema. Of these, 235 were negative in skin test and oral provocation and two showed IgE antibodies to penicilloyl. After retreatment of the test-negative children with penicillin for acute infections, 16% reacted with slight skin reactions or gastrointestinal symptoms, which from the experience described above is unexpectedly high. The reactions were mostly seen in children below 5 years of age, which is unexpected if the reactions were true penicillin allergy (24, 60, 61, 72).

Solley et al. (71) from 1971 to 1978 skin tested 778 patients with previous histories of penicillin allergy using skin testing reagents consisting of benzylpenicillin, ampicillin, methicillin, penicilloyl-polylysine conjugates and the penicilloate of each penicillin, prepared less than 1 week before testing. In contrast to the study by Sullivan et al. (73) described above, in this study Solley et al. showed that 14% of the tested patients exhibited one or more positive skin tests. Addition of methicillin and ampicillin increased the number of positive patients. In view of the discussion about the specificity of the immune responses, this could then mean that in these two studies different numbers of patients had been treated with different types of penicillins. Eight patients with positive skin tests were treated again with penicillin and four of them had reactions. Of the patients with positive skin tests, 27 were treated with cephalosporins and none of them reacted. Of the patients with negative skin tests 4.1% had reactions when treated again with penicillin. Of 151 patients also with negative tests 1.5% had reactions when treated with a cephalosporins. These studies, as well as others, (37, 53, 54, 75), imply that skin test results are valuable in the diagnosis of penicillin allergy, provided the test is carefully standardized and the reagents carefully selected in relation to the history of the patient. When cell-mediated immune reactivity may be involved, epicutaneous testing is proposed worthwhile. Patients with IgE-mediated immediate-type allergy, as well as patients with morbilliform exanthemas, have been shown to respond to such testing (26).

In all studies using skin test reagents, the definition of the antigen is crucial. Whether a multivalent antigen will be formed in an appropriate quantity after injection in the skin during the 15 min latency period until the test results are evaluated, or whether it is already present in the reaction mixture, is not settled. Some theoretical calculations have been performed on this subject (2). After injection of 50 μ l of a 10^{-2} M solution of penicillin intradermally, the amount of conjugated protein carrying penicillin determinants can be theoretically estimated at about 2 μ g of antigen carrying more than two determinants per protein molecule and less than 10^{-5} μ g of molecules carrying more than five determinants per molecule. These amounts should be compared with the theoretically expected amount of 4×10^{-4} μ g of high molecular weight impurities carrying more than five determinants in the penicillin preparation (2). Thus, more efficient eliciting antigens would be expected as contaminants in the penicillin than those formed *in situ* at injection sites.

The Phadebas RAST® (11, 44, 46) and ELISA (23) have been employed in the diagnosis of penicillin allergy. Phadebas RAST results correlate excellently with skin test results using penicilloyl polylysine. The selection of the patients tested has to be considered, however. The *in vitro* tests are more often falsely negative than the skin test, and only about 50% of the patients with suspected penicillin allergy are picked up by *in vitro* testing. This may be explained partly by the fact that penicilloyl has been the only determinant used, no test system having been available employing the so-called minor determinants. It has been claimed that skin testing with only penicilloyl will not pick up the 15–20% of allergic patients detected with minor determinants (70), and similar results would be expected with the RAST.

Recently, Edwards et al. (31) compared benzyl penicillanyl, thiol-linked benzyl penicillenate and thiol-linked penicillamine with penicilloyl in the RAST, using serum samples from 90 patients with a history of penicillin allergy, and factory workers who had reported symptoms

after exposure to penicillin. Of the patients with symptoms after clinical treatment with penicillin two thirds were positive to the penicilloyl determinant and at least one of the minor determinants. One third of them were positive to all determinants. Samples from one fourth of the patients were positive only when the penicilloyl determinant was used, whereas one fourth were negative to the penicilloyl determinant, but positive to some of the new determinants. Some rather strong reactions to the penicillanyl determinant were noted in the patients. Of the penicillin factory workers, a few showed weak responses to penicilloyl or to the penicillenate, penicillamine or penicillanyl, but not to the penicilloyl. Two sera from the 101 controls were also positive to the so-called minor determinants. The explanation for this is not obvious, and must be better evaluated if these determinants are to be used for clinical diagnosis of penicillin allergy. The study indicates the possibility to develop a better *in vitro* diagnostic tool for penicillin allergy.

These results did not confirm those of another recent study (47), where the penicillanyl and penicillamine determinants were used. In the latter study only the penicillamine determinant added some more information compared with the tests performed using the penicilloyl determinant alone. The reason for this discrepancy is most probably to be found in the production of conjugated antigens. The procedures for manufacture seem to be difficult to standardize.

Practical considerations

Both theoretical and experimental data indicate that penicillin preparations containing high molecular weight protein impurities are more immunogenic than preparations without such contaminants. By rather simple means the content of contaminants can be determined and eliminated. Penicillin preparations with such impurities should therefore not be accepted for clinical use.

It has also been shown that penicillin is degraded in the presence of carbohydrates and certain metal ions like iron. A penicillin solu-

tion containing glucose will be more immunogenic due to degradation when stored. Acceptable storage conditions should be better defined, particularly for solutions containing catalyzing substances like glucose and metal ions. Such evaluation should also include the mixtures used for oral administration. These mixtures contain a considerable number of compounds, which seem to result in penicillin-conjugated antigens and possibly also reactive metabolites for oral mixtures. Prevention of such degradation could be accomplished for by microencapsulation or preparation of the mixture immediately before use. By such measures it should be possible to reduce, albeit not eliminate, the number of true allergic reactions to penicillin. However, the use of extremely pure preparations for skin testing would possibly be accompanied by a decreasing number of positive reactions and increasing numbers of falsely negative results due to decreased antigen content and antigen formation *in situ*, even if a battery of relevant structures is used. Well-defined skin test reagents should preferably be produced by the penicillin manufacturers, who could also provide penicillin metabolite determinants for *in vitro* testing, where a highly pure and well-defined penicillin was used, this would provide the means of adequate non-hazardous serodiagnosis of penicillin-allergic individuals. Such reagents and test methods are badly needed, since without establishment of a firm link between an allergic inflammatory reaction and a penicillin-derived antigen, adverse reactions seen during penicillin therapy cannot be classified.

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