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SPECIFICITY FOR BENZYL-PENICILLIN, PENICILLOYL COMPOUNDS, AND BENZYL- PENICILLOATE IN HUMAN ALLERGY TO PENICILLIN

By

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INTRODUCTION

Penicillin allergy is a condition in which disease may be elicited by an immunological reaction to penicillin. Human penicillin allergy may be due to several related chemical radicals, and the allergic reaction may be of the immediate (humoral) or delayed (cellular) type. Moreover, human penicillin allergy shows extremely varied manifestations, from mild urticaria to severe systemic reactions, from mild eczema to severe cases of exfoliative dermatitis. Thus, the term penicillin allergy does not refer to a simple, easily defined disease entity.

In human penicillin allergy of the immediate type, which is the sole subject of the following, many investigations have shown that in 15-20 per cent of the allergic patients a positive cutaneous reaction may be elicited by *crystalline benzylpenicillin* (Fig. 1) (Finke, Grieco, Connell, Smith & Sherman 1965, de Weck & Blum 1965, Redmond & Levine 1967). In the serum of penicillin allergics, furthermore, there are occasionally reagins (skin sensitizing antibodies) which after passive transfer to normal human skin may elicit a typical

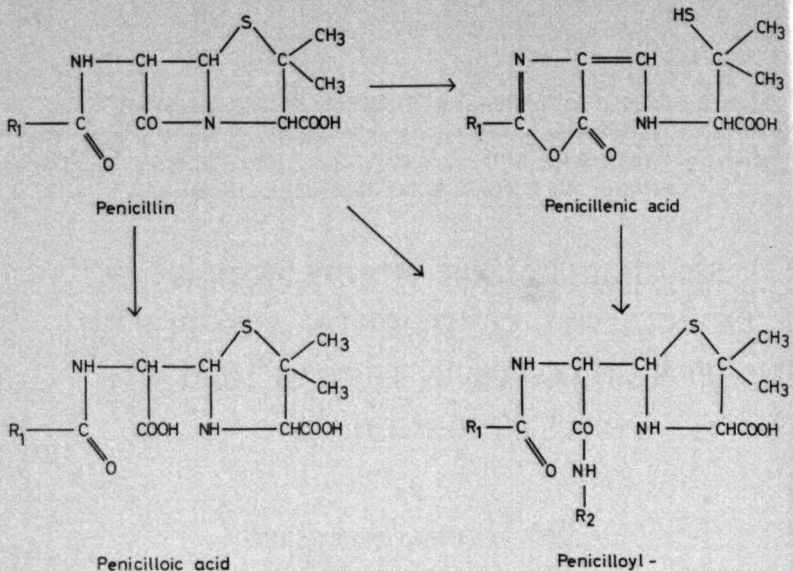


Fig. 1.

wheal-and-flare reaction upon injection of commercial crystalline penicillin (Siegel 1962, van Arsdell, Tobe & Pasnick 1963). Such reagins occur in the serum of 20 per cent of patients with penicillin allergy (Redmond & Levine 1967), or 30 per cent (Pedersen-Bjergaard 1969). The higher frequency of reactions to penicillin in the passive transfer tests than in the cutaneous tests was possibly due to a higher percentage of systemic reactions among the patients studied in the passive transfer tests.

Impurities in commercial penicillin preparations were attributed with significance in penicillin allergy already in early papers (Silvers 1944, McGuire 1946, Bauer 1946). Subsequent investigations have revealed that 35–75 per cent of penicillin allergics give positive cutaneous reaction to *penicilloyl compounds* (Fig. 1), e.g. penicilloyl-polylysine (de Weck 1962, Parker, Shapiro, Kern & Eisen 1962, Simpson 1962, Rytel, Klion, Arlander & Miller 1963, Brown, Price &

Moore 1964). Correspondingly, in passive transfer to normal human skin of serum from penicillin allergics, reactions are obtained with penicilloyl compounds in 25–40 per cent (Redmond & Levine 1967, Pedersen-Bjergaard 1969).

Recently, it has been demonstrated that penicilloyl compounds usually occur as impurities in the commercial penicillin preparations (Batchelor, Dewdney, Feinberg & Weston 1967, Stewart 1967, de Weck, Schneider & Gutersohn 1968), partly as penicilloylated substances of protein nature and partly in the form of low-molecular polymeric compounds. This has revived interest in allergic reactions to impurities and to the possible advantages of purifying commercial penicillin preparations.

Degradation products of penicillin also appear to play a role in eliciting allergic reactions. Thus, penicillin allergics have given positive patch tests to penicillin even after it has been inactivated by penicillinase (Goldman, Friend & Mason 1946). Furthermore, in passive transfer tests with sera from penicillin allergics it has been observed that positive reactions could not be elicited by procainepenicillin unless the penicillin preparation had been heated for some time (Siegel & Coleman 1957).

Benzylpenicilloate, the salt of penicilloic acid (Fig. 1), seems to be quantitatively the most important degradation product of benzylpenicillin *in vivo*. Thus, it is present in considerable quantities in the urine after administration of penicillin (Ullberg 1954), and in several cases it has proved capable of eliciting positive cutaneous reaction in penicillin allergics (Redmond & Levine 1967), and positive reaction in passive transfer tests to normal human skin of serum from penicillin allergics (Redmond & Levine 1967, Pedersen-Bjergaard 1969).

Thus, in human penicillin allergy benzylpenicillin, the penicilloyl radical, and benzylpenicilloate may elicit positive cutaneous reaction and positive reaction in passive transfer tests. The following possibilities of reaction to the 3 chemical compounds exist:

- 1) Independent reactions to 3 specific and different antigens without cross allergy.
- 2) Partial cross allergy between 2 or 3 otherwise different antigens due to the uniform chemical structure of the compounds (Fig. 1). Possibly, only inhibition by one antigen of reactions elicited by one of the others.
- 3) Complete cross allergy, *i.e.* reaction to one and the same antigen structure occurring in 2 or all 3 chemical compounds.

Provided that there are 3 specific antigens, represented by benzylpenicillin, the penicilloyl radical, and benzylpenicilloate without cross allergy, there is furthermore the question:

- 4) Is it always possible in each individual patient upon cutaneous tests and in passive transfer tests to obtain a definite determination of the antigen specificity by the use of commercially available antigen preparations?

Independent specificity for benzylpenicillin must be assumed to occur judging by the results of cutaneous tests in penicillin allergics (*de Weck & Blum 1965, Redmond & Levine 1967*), as some patients give positive reaction only to benzylpenicillin. Passive transfer tests with sera from penicillin allergics to normal human skin support this assumption, as reactions in some sera are elicited by benzylpenicillin as the only antigen (*van Arsdell, Tobe & Pasnick 1963, Redmond & Levine 1967, Pedersen-Bjergaard 1969*).

Independent specificity for the penicilloyl radical seems likely judging from several previous studies. Thus, positive cutaneous reaction to penicilloyl-polylysine commonly occurs in penicillin allergics with negative cutaneous reaction to benzylpenicillin and benzylpenicilloate. Furthermore, positive cutaneous reactions to penicilloyl-polylysine are specifically inhibited by previous injection of the univalent antigen penicilloyl aminocaproate (*Levine & Ovary 1961*). Finally, many penicillin allergics have been found to possess skin-sensitizing antibodies which react only with penicilloyl compounds (*Red-*

mond & Levine 1967, Pedersen-Bjergaard 1969), not with benzylpenicillin or benzylpenicilloate.

Independent specificity for benzylpenicilloate is less substantiated. Cutaneous reactions and passive transfer tests are positive to penicilloate and negative to penicillin and penicilloyl compounds in only a very few penicillin allergics (Redmond & Levine 1967). In addition, very high concentrations of penicilloate (approx. 10^4 times higher than with benzylpenicillin and penicilloyl-polylysine) are required to elicit positive reaction in passive transfer tests to normal human skin (Pedersen-Bjergaard 1969).

Thus, it may be assumed that 2, perhaps 3, different antigens play a role in penicillin allergy and that reaction with these antigens may be elicited by benzylpenicillin, penicilloyl compounds, and benzylpenicilloate. A complete cross allergy, as mentioned under item (3), may presumably be ruled out.

In human penicillin allergy some patients react to more than one of the 3 antigens at the same time. This has been observed as a positive cutaneous reaction with benzylpenicillin as well as penicilloyl-polylysine in 17 out of 120 penicillin allergics (Finke, Grieco, Connell, Smith & Sherman 1965), in 3 out of 80 penicillin allergics (Voss, Redmond & Levine 1966), and in 6 out of 20 penicillin allergics (de Weck, Schneider & Gutersohn 1968). In passive transfer tests too, reaction with more than one of the 3 antigens benzylpenicillin, penicilloyl-polylysine, and benzylpenicilloate may often be obtained in serum from the same patient with penicillin allergy (Redmond & Levine 1967, Pedersen-Bjergaard 1969). Such reactions may be specific, indicating independent hypersensitivity to different antigens. However the results might also be explained by *partial cross allergy* between penicillin, penicilloyl- and penicilloate compounds (item 2), or by *lacking specificity* of the tests used, including the use of impure or unstable antigen preparations (item 4).

A few authors have suggested that penicillin and penicilloate may exert an inhibitory effect upon the induction of penicilloyl specific reactions (Parker, Shapiro, Kern & Eisen

1962, *de Weck*: personal communication), but such inhibition does not ever seem to have been definitely confirmed or rejected in human penicillin allergy.

Cutaneous testing of penicillin allergics with penicillin has been a moot point through many years. Some patients seem to be extremely sensitive to penicillin, intracutaneous tests using fractions of 1 unit of benzylpenicillin having in many cases elicited systemic allergic reaction (*Coleman & Swineford* 1956, *Bierlein* 1956, *Pedersen-Bjergaard* 1967a). Therefore, warnings have been sounded against routine cutaneous testing of penicillin allergics with penicillin (*Siegel & Coleman* 1957).

However, cutaneous tests with penicillin are still being done in the event of a suspicion of penicillin allergy, in particular for research purposes. To this end, commercial penicillin preparations are used, in scratch and intracutaneous tests, usually in concentrations of 100–10,000 units/ml. High penicillin concentrations give a higher frequency of positive intracutaneous reactions in penicillin allergics (*Pedersen-Bjergaard*, to be published), but the question is whether such reactions are invariably penicillin specific.

As already mentioned, commercial benzylpenicillin is regularly contaminated with various penicilloyl impurities. In addition, penicilloyl compounds may be formed in the organism from penicillin, and lastly penicillin is to a marked extent broken down *in vivo* to penicilloate. Theoretically, thus, there ought to be a possibility that reactions to penicilloyl compounds and penicilloate may be elicited by penicillin in cutaneous tests and passive transfer tests.

Comparison of the results of intracutaneous tests using commercial penicillin in increasing concentrations and a determination of the specificity for skin sensitizing antibodies in a group of penicillin allergics gave rise to a presumption that commercial penicillin in a high concentration may elicit penicilloyl-specific intracutaneous reactions (*Pedersen-Bjergaard*, to be published). Furthermore, elicitation of penicilloyl specific passive transfer tests has been reported with large

quantities of commercial penicillin injected intravenously into a normal human recipient (*Pedersen-Bjergaard* 1967b).

In other words, several findings indicate that item (4) cannot be fulfilled, *i.e.* that antigen specificity cannot always be assessed with certainty on the basis of routine diagnostic procedures. This appears to apply particularly when commercial penicillin in a high concentration is used.

The *object of the present study*, therefore, was to ascertain:

I: whether in human penicillin allergy there is different specificity for 2 or 3 antigens represented by benzylpenicillin, penicilloyl-polylysine, and benzylpenicilloate (item 1), or whether the reaction is to one and the same antigen structure (item 3),

II: whether there is partial cross allergy between the 3 antigens within the concentration differences obtainable in practice and whether there is a mutual inhibition of the induction of positive reactions (item 2),

III: whether reaction to one antigen may be elicited by preparations of another antigen; in particular, whether penicilloyl- and penicilloate-specific reactions may be induced by commercial penicillin in a high concentration (item 4).

All the investigations were done by passive transfer tests to human skin using the Prausnitz-Küstner technique (*Prausnitz & Küstner* 1921). This method is well suited for inhibition experiments, as is apparent from the control experiments included in the present study. Furthermore, the use of large quantities of antigen in passive transfer tests does not involve any appreciable risk of a systemic allergic reaction in the normal human recipient as does intracutaneous testing in penicillin allergics.

MATERIALS AND METHODS

Sera from 19 patients with penicillin allergy, all containing skin-sensitizing antibodies—reagins—reacting with at least one of the antigens benzylpenicillin, penicilloyl-polylysine, and benzylpenicilloate (*Pedersen-Bjergaard* 1969) were used in the studies. All sera were drawn and stored under sterile conditions at

—20° C until the experiments could be carried out. Such storage causes little reduction of the reagin titre, even over long periods (*Perlman & Layton 1967*).

Passive transfer tests by the Prausnitz-Küstner technique were performed on 8 non-allergic human recipients, all in good general condition. The recipients were sensitized by intracutaneous injection of 0.1 ml serum from the allergics into several premarked sites on the back. These injections, as reported previously by other authors, were often followed by considerable local wheal-and-flare reactions, but never by other allergic symptoms. The reactions were elicited 24 hours later by injecting 0.05 ml antigen solution intracutaneously into the sensitized sites. Positive reactions were typical wheals having a minimum diameter of 5 mm and surrounded by at least 10 mm erythema, all differing clearly from a control reaction in a non-sensitized site.

No recipient had a history of penicillin allergy, and immediately before each induction of the Prausnitz-Küstner reactions all the recipients had intracutaneous tests on the forearm, far from the sensitized site on the back, by 0.05 ml antigen solution. The result of this test was used as a control test in assessing the Prausnitz-Küstner reactions while at the same time it ensured that the recipient did not show a positive cutaneous reaction to the antigen used. Distant elicitation of Prausnitz-Küstner reactions was often observed on the recipients' back following intracutaneous tests with penicillin on the forearm. All the recipients were good reactors upon passive transfer of reagins, assessed on the basis of the positive reactions obtained in all the experiments.

Reagents

Purified benzylpenicillin as the sodium salt Puripen® (batch H 28/1), (Beecham, Brentford, Middlesex, England).

Sephadex-purified benzylpenicillin as the sodium salt (prep. 680322), freeze-dried immediately after purification on a Sephadex column G 25 (Leo Pharmaceutical Products Trading Ltd., Copenhagen).

Danish commercial crystalline benzylpenicillin as the sodium salt Penicillin Novo® (batch 16* 17) (Novo Industri A/S Copenhagen).

Penicilloyl-polylysine Cilligen® containing 5×10^{-4} M and 1×10^{-5} M penicilloyl radical and less than 0.05 units penicillin/1 ml (Sigma Chemical Company St. Louis U.S.A.).

Benzylpenicilloate as the sodium salt made of Sephadex-purified benzylpenicillin by hydrolysis with sodium hydroxide. This preparation contained 1.7×10^{-1} M benzylpenicilloate and less than 0.05 units penicillin/10 ml (Leo Pharmaceutical Products Trading Ltd., Copenhagen).

RESULTS

Cross Absorption Experiments

Six recipients were sensitized intracutaneously on the back by the Prausnitz-Küstner's technique, each with a panel consisting of 17 different sera from penicillin allergics. 10 of these sera had previously reacted with benzylpenicillin, 11 with penicilloyl-polylysine, and 10 with benzylpenicilloate in passive transfer tests.

24 hours after the sensitization of the recipients 0.05 ml concentrated antigen solution was injected as an inhibitory dose into all the sensitized fields. One of the following solutions was used for each recipient (Table 1: primary injection, large dose):

- (a) Sodium benzylpenicillin (Sephadex-purified Leo preparation) $1.7 \times 10^{-1} \text{ M} = 100,000 \text{ units/ml}$, or
- (b) penicilloyl-polylysine $5 \times 10^{-4} \text{ M}$, or
- (c) sodium benzylpenicilloate $1.7 \times 10^{-1} \text{ M}$.

This elicited in all 6 recipients strong wheal-and-flare reactions in many of the sensitized sites (Fig. 2A).

2-3½ hours later, as soon as all the positive reactions to the large dose of antigen had subsided, a diluted antigen solution, 0.05 ml, was injected intracutaneously into all the sensitized fields. One of the following 3 solutions was used for eliciting reactions (Table 1: secondary injections, small dose):

- (d) Sodium benzylpenicillin (Puripen® or Sephadex-purified Leo preparation) $1.7 \times 10^{-5} \text{ M} = 10 \text{ units/ml}$, or
- (e) penicilloyl-polylysine $1 \times 10^{-5} \text{ M}$, or
- (f) sodium benzylpenicilloate $1.7 \times 10^{-2} \text{ M}$.

Thereafter, all the sensitized sites were read for positive reaction at the end of 5, 10, 15, and 30 minutes (Fig. 2B).

A. After benzylpenicillin 1.7×10^{-1} M, 0.05 ml intracutaneously into all sensitized sites. (Primary injection, large dose).

B. After penicilloyl-polylysine 1×10^{-5} M, 0.05 ml intracutaneously into all sensitized sites. (Secondary injection, small dose).

C. After benzylpenicilloate 1.7×10^{-2} M, 0.05 ml intracutaneously into all sensitized sites. (Secondary injection, small dose).

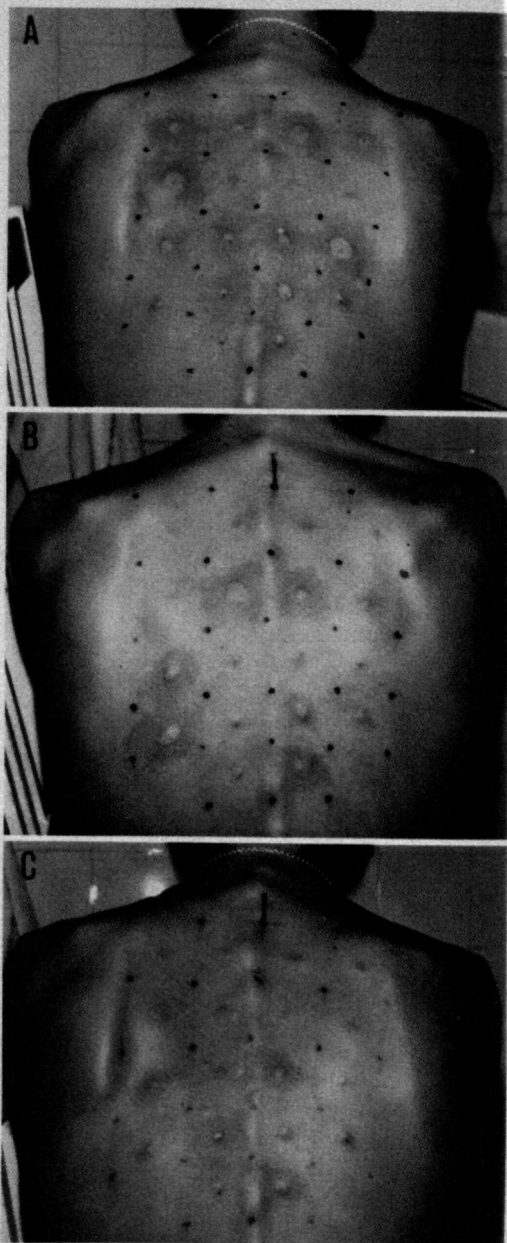


Fig. 2.

Cross absorption experiments in passive transfer tests on a recipient showing positive reactions after successive injections of antigens. The sensitized sites are indicated by black dots in the circumference.

TABLE 1

Cross Absorption Experiments in Passive Transfer Tests Using Human Skin-Sensitizing Antibodies Showing 3 Different Specificities, Represented by Benzylpenicillin, the Penicilloyl Radical, and Benzylpenicilloate.

Primary injection (large dose) for inhibition into all sensitized sites	Secondary injections (small dose) a few hours later into all sensitized sites			
	Benzyl- penicillin 1.7×10^{-5} M	Penicilloyl- polylysine 1×10^{-5} M	Benzyl- penicilloate 1.7×10^{-2} M	
Benzylpenicillin 1.7×10^{-1} M		7 ⁺ 4 ⁻ 10 ⁺ 1 ⁻	7 ⁺ 3 ⁻ 8 ⁺ 2 ⁻	
Penicilloyl-polylysine 5×10^{-4} M	8 ⁺ 2 ⁻ 9 ⁺ 1 ⁻		10 ⁺ 6 ⁻ 9 ⁺ 1 ⁻	
Benzylpenicilloate 1.7×10^{-1} M	8 ⁺ 2 ⁻	11 ⁺ 0 ⁻ 10 ⁺ 1 ⁻		

The results are expressed as positive and negative reactions on the same recipient to the secondary injections (small dose) among all sera with specificity to the secondarily injected antigens.

After another 2 or 3 hours, when all positive reactions had subsided, 0.05 ml diluted antigen solution (d), (e), or (f) was again injected intracutaneously into all sensitized fields (Table 1: secondary injections, small dose), and the reactions were read again (Fig. 2C).

The 3 subsequent injections of antigen administered to the same recipient at a few hours' intervals were always given with different antigen from injection to injection. In addition, the sequence of the individual antigens was varied. Thus, the reactions were induced according to the following schedule:

Recipient No.	Sequence of antigen injections		
1	(a)	(e)	(f)
2	(a)	(f)	(e)
3	(b)	(d)	(f)
4	(b)	(f)	(d)
5	(c)	(d)	(e)
6	(c)	(e)	

TABLE 2
Penicilloyl-Specific Reactions Elicited in Passive Transfer Tests after Intracutaneous Injection of Commercial and Sephadex-Purified Benzylpenicillin.

Intracutaneous injection into all sensitized sites with	Sera from penicillin allergics used for passive transfer tests, all containing penicilloyl-specific skin sensitizing antibodies										Recipients
	Penicillin preparation	Concentration of penicillin	EC	KAH	ALJ	EJN	EMT	EJ 2	KU	EGC	HP
Commercial Benzylpenicillin	10 units/ml	10 units/ml	—	—	—	—	—	—	—	—	—
	100 units/ml	100 units/ml	—	—	—	+	+	—	—	—	—
	1000 units/ml	1000 units/ml	—	—	—	+	+	—	+	—	—
(16* 17)	10,000 units/ml	10,000 units/ml	—	+	—	+	—	+	+	+	—
	100,000 units/ml	100,000 units/ml	—	—	—	+	+	+	+	+	+
Sephadex-purified Benzylpenicillin (680322)	100,000 units/ml	100,000 units/ml	—	—	—	+	—	—	—	—	—

I.H.

H.K.

P.H.

The result of the cross absorption experiments may be seen from Table 1. In spite of preceding inhibition in all sensitized sites injecting one of the 3 antigenic solutions: purified benzylpenicillin, penicilloyl-polylysine, or benzylpenicilloate in highest obtainable concentration—large dose—it was possible in the case of most of the sera to elicit a positive Prausnitz-Küstner reaction as previously with the other two antigens in high dilution—small dose (Fig. 2B and C).

A single positive penicilloyl-specific reaction was observed in serum E.J.N. in recipient P.H. after administration of Sephadex-purified penicillin in concentrated solution (Table 2). Apart from this reaction the large doses of the 3 antigens were never followed by new positive reactions for the sera studied compared with previous results elicited for the same sera with much smaller quantities of antigen (*Pedersen-Bjergaard* 1969). Thus, there was no indication of cross allergy between the 3 radicals with the antigen solutions used in the cross absorption experiments.

Induction of Penicilloyl-Specific Reactions with Commercial Penicillin

Three recipients, one identical with those mentioned above, were sensitized by the Prausnitz-Küstner technique, each with a panel of 7–9 sera from penicillin allergics. In previous investigations of these sera, 5 had reacted only with penicilloyl-polylysine, 4 with penicilloyl-polylysine as well as benzylpenicilloate, but none with benzylpenicillin. 24 hours after the sensitization two of the recipients, I.H. and H.K., were injected subsequently at 20 min.—2 hour-intervals with Danish commercial penicillin (Novo batch 16* 17) in increasing concentration, 0.05 ml intracutaneously into all the sensitized sites. These injections elicited an increasing number of positive reactions at penicillin concentrations of from 100 units/ml to 100,000 units/ml (Table 2). The use of Sephadex-purified penicillin in the highest concentration, 100,000 units/ml, elicited, in a corresponding experiment on a third recipient

(P.H.), a positive reaction with only one penicilloyl-specific serum, E.J.N. Like the other recipients, P.H. was a good reactor with strong positive reactions to penicillin-specific transfer tests (Fig. 2A).

All positive reactions elicited by commercial benzylpenicillin in penicilloyl-specific sera were totally inhibited by preceding intracutaneous injection into the sensitized sites of 0.05 ml penicilloyl-polylysine 5.0×10^{-4} M.

Control Experiments

Successive injections at a few hours' intervals of a specific antigen in increasing concentration into the same fields sensitized with reagin-containing sera elicited repeated positive reactions. But if an equal dose of antigen was used for successive injections, positive reactions usually occurred only after the first injection; only exceptionally did a questionable or very weak reaction appear after the 2nd injection. If a large dose of antigen was first injected into sensitized fields, a new reaction could never be elicited by subsequent injections of smaller doses of antigen.

DISCUSSION

The *cross absorption experiments* (Table 1) are illustrated schematically in Fig. 3. The sides of the squares represent, on a logarithmic scale the injected doses of antigen. The highest possible antigen concentration was used in the primary injection (large dose). Benzylpenicillin and benzylpenicilloate, 1.7×10^{-1} M, cause slight local irritation, and higher concentrations may result in necrosis at the site of injection. Penicilloyl-polylysine could not be procured in a concentration higher than 5×10^{-4} M. At the secondary injection (small dose) the author used the smallest amount of antigen which can definitely elicit maximum reactions in passive transfer tests. As previously mentioned (Pedersen-Bjergaard 1969), maximal reactions to benzylpenicillin and penicilloyl-polylysine

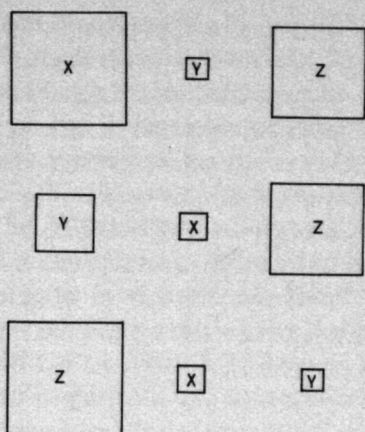


Fig. 3.

Theory of cross absorption experiments in passive transfer tests using skin-sensitizing antibodies from penicillin allergies. The squares represent, on a logarithmic scale, the quantities of antigen injected successively into each sensitized field.

X = benzylpenicillin.

Y = penicilloyl-polylysine.

Z = benzylpenicilloate.

may be elicited in passive transfer tests by small amounts of antigen. On the other hand, reactions to benzyl-penicilloate cannot be elicited until the penicilloate solution is of the concentration 1.7×10^{-2} M. In the present studies, therefore, the penicilloate concentration could only be varied by a factor 1:10 between small and large dose.

Is there a specificity for 3 different antigens in penicillin allergy, and are these antigens represented by pure benzyl-penicillin, the penicilloyl radical, and benzylpenicilloate?

Let us first consider the relation $x = \text{benzylpenicillin}$ versus $y = \text{the penicilloyl radical}$. In the experiment shown in Fig. 3, top line, a considerable dose of x was first injected into a field sensitized with reagin containing serum from an allergic person. If, thereafter, a reaction can be elicited in the same field by a subsequent injection of a small amount of y , the latter must (if the reactions to x and y are due to a com-

mon antigen) contain more antigen than the large dose of x—cf. the results of the control experiments. In the experiment illustrated in Fig. 3, 2nd line, a somewhat larger quantity of y (than in the preceding experiment) was injected first. Presupposing still that reactions to x and y are due to a common antigen, a subsequent injection, into the same sensitized field, of a small quantity of x ought not to be able to elicit a reaction. The small quantity of x must contain too little of the common antigen. But if the small dose of x in the latter experiment does cause a positive reaction, there must be 2 different specificities: x and y. A similar reasoning must apply to specificity for x versus z (benzylpenicilloate) and for y versus z.

The results obtained in the cross absorption experiments (Table 1) show, in conformity with the above considerations, that the serum from penicillin allergics contains skin-sensitizing antibodies, reagins, which react with at least 3 specific and different antigens. Reactions to these antigens may be elicited by purified benzylpenicillin, penicilloyl-polylysine, and benzyl-penicilloate (cf. item 1 in the introduction).

By injection of the concentrated antigen solutions (primary injection) into the sensitized fields it was not possible to demonstrate signs of cross allergy between the 3 radicals (in conformity with item 1 and at variance with item 2 in the introduction). The only reaction which might arouse a suspicion of such cross allergy occurred in serum EJN in recipient P.H. after Sephadex-purified penicillin. This reaction should presumably rather be ascribed to formation of penicilloyl compounds in the penicillin preparation used, either *in vivo* or *in vitro*.

There are factors of some uncertainty associated with passive transfer tests, particularly when using sera with a low titre of reagins. Moreover it is well-known that repeated elicitation of passive transfer tests in the same site causes an increasing reduction of the reactions (*Bowman & Walzer 1953*). Accordingly, the results of the cross absorption experiments do not indicate significantly mutual inhibition between peni-

cillin, the penicilloyl radical, and penicilloate (at variance with item 2 in the introduction). In spite of preceding inhibition, administering each individual antigen in a large dose, reactions could be elicited in the case of most or all sera with the other antigens in a small dose.

The experiments done to elicit penicilloyl-specific passive transfer tests by commercial benzylpenicillin (Table 2) show that commercial benzylpenicillin can induce such positive reactions (cf. item 4 in the introduction). With the Danish penicillin preparation a positive reaction for the first 2 sera (EJN and EMT) was induced already at a penicillin concentration of 100 units/ml, and at a concentration of 100,000 units/ml a reaction was induced in 6 of the 9 sera. More recent investigations indicate that entirely corresponding results are obtainable by other commercial penicillin preparations (*Pedersen-Bjergaard*, to be published).

All the reactions elicited by the commercial penicillin were penicilloyl-specific, as they could be inhibited by penicilloyl-polylysine. The reactions were attributed mainly to penicilloyl contamination of the penicillin preparation, as Sephadex-purified benzylpenicillin in the highest concentration elicited a definitely positive reaction in the case of only one serum (EJN). Penicilloate-specific reactions were never elicited by commercial penicillin.

The results are fully compatible with previous reports stating that purified benzylpenicillin elicits fewer positive cutaneous reactions than commercial benzylpenicillin in penicillin allergies (*Stewart 1967, Knudsen, Robinson, Croydon & Tees 1967*), and that commercial benzylpenicillin in a high dose administered intravenously may elicit penicilloyl-specific passive transfer tests (*Pedersen-Bjergaard 1967b*).

The results indicate that in the future penicillin-specific cutaneous tests and reagin studies should be performed with specially purified penicillin preparations or commercial penicillin in a concentration so low that penicilloyl-specific reactions cannot be elicited. Complete agreement between results obtained by low concentration of commercial and by highly

purified benzylpenicillin has been demonstrated in a previous communication (*Pedersen-Bjergaard* 1969). Previously reported penicillin-specific reactions elicited by commercial benzylpenicillin in cutaneous tests and passive transfer tests must be presumed to have been penicilloyl-specific in some cases.

SUMMARY

According to previous investigations, including cutaneous tests and passive transfer tests, it seemed likely that in human penicillin allergy there were at least 2, perhaps 3 specific and different antigens, represented by benzylpenicillin, the penicilloyl radical, and benzylpenicilloate.

Penicilloyl compounds are formed from penicillin by a reaction with proteins and occur also as contamination of the commercial penicillin preparations. Benzylpenicilloate is quantitatively the most important degradation product of benzylpenicillin *in vivo*.

Reaction to the 3 antigens benzylpenicillin, the penicilloyl radical, and benzylpenicilloate was studied in further detail in passive transfer tests by cross absorption experiments. Passive transfer tests to human skin were performed by a panel of reagin-containing sera from penicillin allergics. Thereafter, reactions were elicited by successive injections into the same sites of purified benzylpenicillin, penicilloyl-polylysine, and benzylpenicilloate in varied sequence. First, an antigen was injected in the highest possible concentration and thereafter the other two in the lowest concentration which previously had proved able to elicit positive reactions in passive transfer tests.

The results confirm that the serum of penicillin allergics contains skin-sensitizing antibodies with independent specificity for 3 antigens represented by benzylpenicillin, the penicilloyl radical, and benzylpenicilloate, as slight or no inhibition was observed in the cross absorption experiments. After the high antigen concentrations, moreover, there were no definite signs of cross allergy between benzylpenicillin, the peni-

cilloyl radical, and benzylpenicilloate, and no signs of mutual inhibition of the induction of positive reactions.

In passive transfer tests with serum from penicillin allergics, penicilloyl-specific reactions could be elicited by intracutaneous injection of commercial penicillin in a sufficiently high concentration. This indicates that in future reagin studies, and presumably also in cutaneous tests on suspected penicillin allergics, specially purified penicillin preparations should be used—or else the tests should be carried out by the use of a sufficiently low concentration of commercial penicillin. Only by doing this will it be possible to avoid simultaneous induction of penicilloyl-specific reactions.

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REFERENCES

- van Arsdel, P. P., Tobe, A. D. & Pasnick, L. J.*: *J. Allergy* 34, 526, 1963.
Batchelor, F. R., Dewdney, J. M., Feinberg, J. G. & Weston, R. D.: *Lancet* I, 1175, 1967.
Bauer, G. H.: *Arch. Dermat. & Syph.* 54, 292, 1946.
Bierlein, K. J.: *Ann. Allergy* 14, 35, 1956.
Bowman, K. L. & Walzer, M.: *J. Allergy* 24, 126, 1953.
Brown, B. C., Price, E. V. & Moore, M. B.: *J.A.M.A.* 189, 599, 1964.
Coleman, W. M. P. & Swineford, O.: *Virginia M. Monthly* 83, 6, 1956.
Finke, S. R., Grieco, M. H., Connell, J. T., Smith, E. C. & Sherman, W. B.: *Am. J. Med.* 38, 71, 1965.
Goldman, L., Friend, F. & Mason, L. M.: *J.A.M.A.* 131, 883, 1946.
Mc. Guire, J. A.: *Arch. Dermat. & Syph.* 53, 31, 1946.
Knudsen, E. T., Robinson, O. P. W., Croydon, E. A. P. & Tees, E. C.: *Lancet* I, 1184, 1967.

- Lewine, B. B. & Ovary, Z.: *J. exp. Med.* 114, 875, 1961.
- Parker, C. W., Shapiro, J., Kern, M. & Eisen, H. N.: *J. exp. Med.* 115, 821, 1962.
- Pedersen-Bjergaard, J.: *Acta Allergol.* 22, 299, 1967a.
- Pedersen-Bjergaard, J.: *Acta Allergol.* 22, 466, 1967b.
- Pedersen-Bjergaard, J.: *Acta Allergol.* 24, 57, 1969.
- Pedersen-Bjergaard, J.: to be published.
- Perlman, F. & Layton, L. L.: *J. Allergy* 30, 205, 1967.
- Prausnitz, C. & Küstner, H.: *Centralbl. f. Bakt.* 86, 160, 1921.
- Redmond, A. P. & Lewine, B. B.: *J. Allergy* 39, 51, 1967.
- Rytel, M. W., Klion, F. M., Arlander, T. R. & Miller, L. F.: *J.A.M.A.* 186, 894, 1963.
- Siegel, B. B. & Coleman, M.: *J. Allergy* 28, 264, 1957.
- Siegel, B. B.: *J. Allergy* 33, 349, 1962.
- Silvers, S. H.: *Arch. Dermat. & Syph.* 50, 328, 1944.
- Simpson, W. G.: *Proc. XII Int. Congr. dermat. Washington 1962*, *Excerpta medica fundat.* p. 897.
- Stewart, G. T.: *Lancet* I 1177, 1967.
- Ullberg, S.: *Acta Radiolog. suppl.* 118, 1954.
- Voss, H. E., Redmond, A. P. & Lewine, B. B.: *J.A.M.A.* 196, 679, 1966.
- de Weck, A. L.: *Dermatologica* 125, 283, 1962.
- de Weck, A. L.: personal communication 1968.
- de Weck, A. L. & Blum, G.: *Int. Arch. Allergy* 27, 221, 1965.
- de Weck, A. L., Schneider, C. H. & Gutersohn, J.: *Int. Arch. Allergy* 33, 535, 1968.

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