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POSSIBLE ADVANTAGES
OF HIGHLY PURIFIED PENICILLIN IN
AVOIDING PENICILLOYL-SPECIFIC
SENSITIZATION AND ALLERGIC REACTIONS*

*Evaluation Based on Passive Transfer Tests
to Normal Human Skin Using Penicilloyl-Specific
Skin-Sensitizing Antibodies*

By

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INTRODUCTION

Penicillin allergy is an important form of drug allergy in man, since its incidence is high and since it may give rise to life-threatening reactions. In addition, therapeutic problems may arise in choosing antibacterial treatment in the event of infectious diseases in patients with penicillin allergy.

A history of *penicillin allergy* among the populations of the western world is of a fairly constant frequency, ranging from 1.8 per cent to 5.2 per cent (43, 47, 13, 16, 29, 22, 28, 36, 40) (Table 1). However, such presumed penicillin allergics most often tolerate penicillin therapy at a later date (51, 17) without exhibiting further allergic reactions. In other cases subsequent administration of penicillin elicits another allergic

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TABLE 1
History of Penicillin Allergy in Patient and Soldier Materials.

No. of persons questioned	Category of persons	Author	Year	Incidence of previous penicillin allergy per cent
3,275	Soldiers	Sherwood et al. (43)	1957	5.2
1,365	Various patients	Smith (47)	1957	4.7
13,089	Soldiers	Mc. Farland (13)	1958	1.8
7,390	Various patients	Foged (16)	1958	3.0
1,800	Soldiers	Olsen (29)	1958	3.6
2,585	Various patients	Lassen (22)	1959	3.2
2,600	Soldiers	Miller (28)	1963	5.0
1,922	Various patients	Peltonen et al. (36)	1963	2.6
1,022	Soldiers	Rytel et al. (40)	1963	4.3

reaction which may prove fatal. Thus, 30 per cent of persons who have died of allergic penicillin reaction (3, 7) had a history of previous penicillin allergy.

The frequency of allergic reactions observed during and after penicillin therapy in persons with no past history of penicillin allergy varies within wide limits. The stated rates range from 0.2–6.5 per cent (45, 9, 46, 59, 43, 13, 1, 11, 28, 36, 40, 19) (Table 2). The rate also varies in apparently comparable materials, for instance between 0 and 8.7 per cent in a W.H.O. study comprising 2.3 million penicillin-treated venereal patients (60). However, there is evidence to indicate that the nature of the penicillin preparation (46), the number of doses administered (43), the mode of administration (2), and in particular the size of the doses (43, 13, 11) play a decisive role in the frequency of allergic reactions occurring during penicillin therapy.

TABLE 2

Allergic Reactions Observed in Connection with Penicillin Therapy.

No. of persons treated	Category of patients	Author	Year	Incidence of allergic reactions per cent
16,345	Venereal	Smith et al. (45)	1954	0.7
2,913	Soldiers	Brooks & Moe (9)	1956	0.9
6,504	Venereal	Smith et al. (46)	1956	0.2-0.5
6,140	Various diseases	Wellman & Corr (59)	1956	0.4-0.9
3,275	Soldiers	Sherwood et al. (43)	1957	3.1-6.1
12,858	Soldiers	Mc. Farland (13)	1958	1.1-1.7
6,832	Various diseases	Andersen (1)	1959	1.3
35,496	Venereal	Brown et al. (11)	1961	0.5-6.5
2,600	Soldiers	Miller (28)	1963	1.0
1,873	Various diseases	Peltonen et al. (36)	1963	0.3
825	Soldiers	Rytel et al. (40)	1963	1.2
95	Subac. bact. endocard.	Grieco et al. (19)	1964	5.3

Other factors too contribute to the extremely varying results. The different care by which patients with previous penicillin allergy are excluded from further penicillin therapy and the doctor's individual evaluation of the clinical signs unless cutaneous tests and provocation experiments are performed. Some authors (59), therefore, make a distinction between definite and more doubtful allergic reactions to penicillin. The uncertainty of the clinical diagnosis of penicillin allergy may be illustrated by the fact that a diagnosis of penicillin allergy has been presumed in several cases in persons who are being treated with a penicillin placebo (6). An allergic reaction occurring during penicillin therapy need not be caused by the penicillin. The patients often receive other drugs at the same

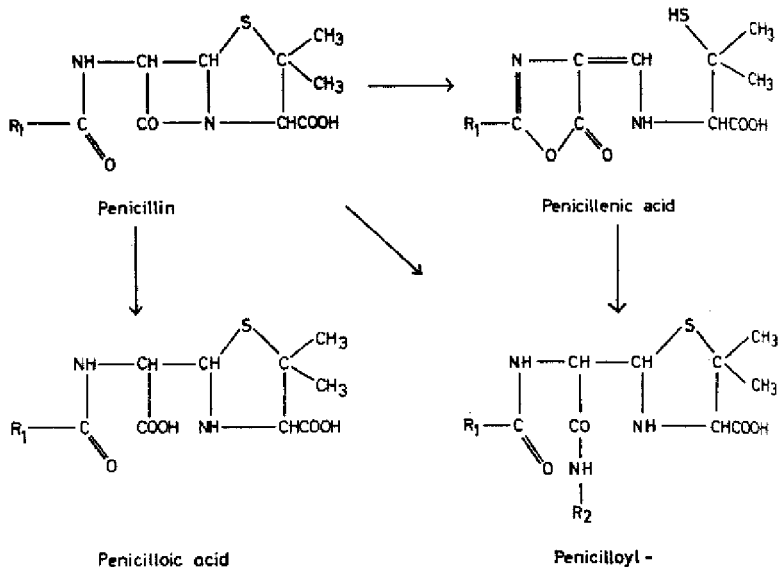


Fig. 1.

time, drugs which may, like *e.g.* foods, cause allergic symptoms.

Serious (life-threatening) allergic reactions to penicillin make up 5–10 per cent of all cases in major analyses (45, 13, 22, 1, 11). In one study (58) more than 30 per cent serious reactions occurred. The mortality of penicillin therapy has been reported to be 1 in 70,000 and 1 in 93,000 penicillin-treated patients in an inquiry to venereological clinics (60, 45). A considerably lower rate, *i.e.* around 1 in 7 million single doses of penicillin, may be calculated from the figures reported in another large material from general practitioners and hospitals in U.S.A. (58). In Denmark 1 death has been recorded in 3–4 million single doses of penicillin (20).

In human penicillin allergy of the immediate (humoral) type, to which the great majority of cases of penicillin allergy have to be assigned, at least 3 different antigens are operative. These antigens are represented by *penicillin*, *penicilloate* (the salt of penicilloic acid), and the *penicilloyl radical* (Fig. 1).

Allergic reactions induced by apparently pure penicillin have been known almost since penicillin was introduced into clinical use. Frequently, these are serious reactions elicited by very small quantities of penicillin. In the early sixties the assumption that penicillenic acid might be the antigen in penicillin allergy (23, 54) led to the detection of the penicilloyl compounds. These compounds may form by a reaction between penicillenic acid or the penicillin molecule itself and proteins, and they occupy a central position in penicillin allergy (24, 30, 31). Penicilloyl-specific allergic reactions are clinically often mild reactions. Finally, penicilloate, a degradation product of penicillin, also appears to play a role in penicillin allergy.

That reactions to these 3 chemical compounds—penicillin, the penicilloyl radical, and penicilloate—occur in human penicillin allergy has been demonstrated by cutaneous tests on penicillin allergics (37) and by studying skin-sensitizing antibodies—reagins—in the serum of penicillin allergics (37, 34). Cross absorption experiments in passive transfer tests using the 3 compounds have shown with certainty that there is a question of 3 specific antigens, and within wide concentration ranges there have been no definite signs of cross allergy between the 3 antigens or of a mutual inhibition of the induction of positive reactions (35).

During systemic penicillin therapy the person treated is subjected to a massive and generalized exposure to penicillin, and such exposure may elicit an allergic reaction showing penicillin specificity. The nature of the penicillin preparation appears to be of less importance in eliciting the penicillin-specific reactions, several investigations having shown a marked mutual cross allergy between the various penicillins (38, 49, 42, 18).

Penicillin is broken down *in vivo* int. al. into penicilloate by hydrolysis of the beta lactam ring. Thus, penicilloate is demonstrable in the urine of persons treated with penicillin (52). It has not been elucidated, however, whether sufficient penicilloate forms *in vivo* during penicillin therapy to elicit an

allergic reaction. High penicilloate concentrations are required to elicit penicilloate-specific passive transfer tests (34).

The possibilities of eliciting penicilloyl-specific allergic reactions in penicillin therapy have also not been fully elucidated. As is apparent from the following, commercial penicillins are regularly contaminated by small quantities of penicilloyl compounds. The extent of this contamination is an important factor in whether or not a penicilloyl-specific allergic reaction is elicited during penicillin therapy (33).

In cutaneous tests and passive transfer tests for penicilloyl-specific reactions, optimal results are obtained by succinylated penicilloyl-polylysine containing 20–100 lysine residues per molecule (26).

Allergic reaction to penicilloyl compounds is quantitatively the most important factor in penicillin allergy. Thus, 35–75 per cent of penicillin allergics show positive cutaneous reaction to penicilloyl-polylysine (31, 55, 44, 40, 10). More rarely a positive cutaneous reaction to penicillin (12, 14, 56) and to penicillin as well as penicilloate (53) may be obtained. Corresponding findings are made in searching for skin-sensitizing antibodies—reagins—in the serum of penicillin allergics (34). In such investigations too penicilloyl-specific reactions show the highest incidence.

However, a positive cutaneous reaction to the penicilloyl radical does not occur exclusively in patients with known penicillin allergy. 4–7.5 per cent of randomly selected persons without a history of penicillin allergy show positive cutaneous reaction to penicilloyl-polylysine (44, 55, 40, 10). As might be expected, positive cutaneous reaction is far more common in persons who have previously been treated with penicillin than in those who have never received penicillin.

After one injection of penicillin to 614 patients with negative cutaneous reaction to penicilloyl-polylysine 8.2 per cent developed a positive cutaneous reaction to penicilloyl-polylysine (40). This shows that penicillin therapy must entail an exposure to penicilloyl compounds and that these compounds are heavily sensitizing.

The antigenic properties of the penicilloyl radical have also been confirmed in another way. By a particularly sensitive technique agglutinating antibodies of the γ -G and γ -M type with specificity for the penicilloyl radical have been demonstrated in 97 per cent of randomly selected human sera (27). However, these serological findings bear no direct relation to reagin-mediated penicillin allergy.

Persons with "chance demonstration" of a positive cutaneous reaction to penicilloyl-polylysine usually tolerate penicillin therapy, only 5-12 per cent showing clinical signs of allergic reaction (44, 40, 10). Even a patient who responded by a severe systemic allergic reaction to intracutaneous testing with penicilloyl-polylysine proved to stand penicillin therapy without complaints (39).

A central question in evaluating the role of the penicilloyl radical in penicillin allergy is: In which way is the patient on penicillin therapy exposed to penicilloyl compounds, and in which quantities do such compounds occur?

It has previously been assumed that patients on penicillin medication are exposed to considerable quantities of penicilloyl compounds. Such compounds form by direct reaction between penicillin and amines, including proteins, at a highly alkaline pH (25, 32), but the reaction may take place also under the physical-chemical conditions encountered *in vivo* (4). Formation of penicilloyl may occur also in reactions between penicillin and more low-molecular compounds, such as monosaccharides, disaccharides, or glycerol (41). Thus, the pre-suppositions appear to be present for an *in vivo* formation of penicilloyl which may elicit an allergic reaction.

In addition, penicilloyl compounds occur regularly as contamination in the commercial crystalline penicillin preparations (5, 21, 50), partly in the form of penicilloyl-containing compounds of protein nature and partly as di- and polymeric compounds (5, 21, 57). The protein compounds are presumably contamination from the culture media used in the manufacture of the commercial penicillin, while the di- and polymeric compounds may form spontaneously from pure penicillin.

While some authors (5, 50) attach most importance to the contamination of the commercial penicillin preparations with penicilloyl impurities of protein nature, others (57) have found evidence, based upon quantitative penicilloyl determinations, that the formation of dimeric and polymeric penicilloyl compounds plays a much greater role in eliciting penicilloyl-specific allergic reactions.

By dialysis and gel filtration it has been attempted to rid commercial penicillins of penicilloyl impurities in the hope of thereby reducing or abolishing the patients' exposure to penicilloyl compounds during penicillin therapy.

Comparisons of cutaneous tests with purified and with commercial penicillin in penicillin allergies have given varying results. In some investigations (21, 50) fewer positive cutaneous reactions have occurred with purified than with commercial penicillin, presumably because penicilloyl-specific reactions are less apt to be elicited by pure penicillin. Others (57) have reported almost as many positive cutaneous reactions with sephadex-purified penicillin as with non-purified commercial penicillin.

AUTHOR'S PREVIOUS INVESTIGATIONS

Exposure to penicilloyl compounds during penicillin therapy was studied in passive transfer tests to human skin of penicilloyl-specific skin-sensitizing antibodies (33).

A non-penicillin allergic recipient was first sensitized locally with a panel of sera from penicillin allergies, all containing penicilloyl-specific skin-sensitizing antibodies. Each sensitized field represents, in the abstract, a penicillin allergic "en miniature". During subsequent penicillin treatment of the recipient a systemic exposure to penicilloyl compounds may be studied by the allergic reaction locally in each sensitized field. The study is carried out "*in vivo*" during penicillin therapy and simultaneously for several penicillin allergies (represented by the respective serum samples). In addition, there is no major risk of eliciting a serious allergic reaction by this investigation.

By this method no signs of penicilloyl-specific reactions were demonstrable in the sensitized fields after treatment with commercial penicillin, 2 mega units i.v. But if the recipients were treated with commercial penicillin, 20 mega units i.v., strongly positive penicilloyl-specific reactions were elicited in a few minutes in a number of the sensitized fields.

The inhibitory effect upon penicilloyl-specific reactions was studied, both immediately after an infusion of 20 mega units commercial penicillin and after longer-lasting penicillin therapy using the same total dose. An almost identical inhibition of penicilloyl-specific passive transfer tests was observed in both forms of treatment. In other words, there were no signs of a gradual and slow formation of penicilloyl compounds *in vivo* during penicillin therapy.

Penicillin from which high-molecular penicilloyl compounds had been removed by dialysis was administered intravenously in a high dosage (20 mega units) to a locally sensitized recipient. This elicited only a faintly positive penicilloyl-specific reaction with a single serum. In all sensitized fields, however, massive inhibition to subsequent elicitation of reactions with penicilloyl-polylysine was observed. This specific inhibition was attributed to the occurrence of low-molecular penicilloyl compounds in the dialysed penicillin preparation used.

OBJECT OF PRESENT STUDY

The object of the present study was (a) to extend the above investigations performed with Danish commercial penicillin, using also British and American commercial penicillin, (b) to assess a commercially available purified penicillin (Purapen®) and (c) to ascertain whether purification of penicillin by sephadex fractionation (50) is more effective than purification by simple dialysis. Finally (d) to elucidate whether systemic penicillin therapy entails exposure to penicilloate in such quantities that a penicilloate-specific allergic reaction may be elicited.

METHOD AND MATERIALS

Passive transfer tests were performed on 20 youngish, non-allergic human recipients in a good general condition. Procedure, precautions, and reading of the reactions have been described in previous papers (33, 34, 35). The recipients were sensitized passively to the penicilloyl radical or to penicilloate in pre-marked fields on the back by intracutaneous injections of 0.1 ml reagin-containing serum. 24 hours after the sensitization the recipients received sodium benzylpenicillin, 20 mega units i.v. Immediately before use the penicillin powder was dissolved in 200 ml sterile 5 per cent glucose solution and was infused intravenously in 5-10 minutes.

The sensitization was performed by 9 sera from penicillin allergies containing penicilloyl-specific and 7 sera containing penicilloate-specific skin-sensitizing antibodies (34). Penicillin specificity did not occur in the penicilloyl-specific sera, and positive penicilloate-specific reactions were never observed after administration of penicillin. Serum KÄH contained, in addition to the named specificities, also reagins to a not yet clarified penicillin antigen. All experiments, moreover, included penicillin-specific reference sera to control the suitability of the recipient for passive transfer tests.

All the recipients exhibited highly positive wheal-and-flare reaction upon intracutaneous injection of histamine 0.01 per cent and negative reactions to the antigen solutions injected into non-sensitized fields.

The following preparations were used for eliciting passive transfer tests:

- (1) Danish commercial crystalline benzylpenicillin as the sodium salt, Penicillin Novo®, lots 1×47 , 8×36 , and 16×17 (the penicillin preparation generally used in the department where the investigations were performed) (Novo Industri A/S, Copenhagen).
- (2) British commercial crystalline benzylpenicillin as the sodium salt, buffered Crystapen®, lot 7693/1 (Glaxo Laboratories Ltd., Greenford, Middlesex, England).
- (3) American commercial crystalline benzylpenicillin as the sodium salt, buffered Sodium-Penicillin G Squibb, lots 8 A 898 and 8 E 619 (E. R. Squibb & Sons Inc., New York).
- (4) British commercial, but specially purified benzylpenicillin, Purapen®, lot H 28/1 (Beecham Research Laboratories, Brentford, Middlesex, England).
- (5) Dialysed benzylpenicillin as the sodium salt Leo, lots 670928 and 671012 (Leo Pharmaceutical Products Trading Ltd., Copenhagen).
- (6) Sephadex-purified benzylpenicillin as the sodium salt, Leo lots 671121, 680322, and 681022. The preparations had been purified in aqueous solution on sephadex G 25 and immediately after freeze-dried (Leo Pharmaceutical Products Trading Ltd., Copenhagen).
- (7) Penicilloyl-polylysine "Cilligen"® containing 1×10^{-5} and 50×10^{-5} M penicilloyl compounds (Sigma Chemical Company, St. Louis, Missouri, U.S.A.).

- (8) Sodium benzylpenicilloate made of sephadex-purified benzylpenicillin by hydrolysis with NaOH at pH 11. Penicilloate concentration 1.7×10^{-3} M, penicillin content less than 0.05 unit/10 ml (Leo Pharmaceutical Products Trading Ltd., Copenhagen).

Preparations (1)–(4) were randomly selected commercial preparations which were stored prior to the investigations in a cool, dark place.

Preparations (5)–(7) were stored in the refrigerator at 4° C, and preparations (5) and (6) were used a few days after they had been manufactured.

Preparation (8) was kept at –20° C until use.

RESULTS

The results in respect to eliciting visible positive penicilloyl-specific passive transfer tests by various penicillin preparations are given in Table 3. Each horizontal column represents the results in the various sera in one recipient for one penicillin preparation. Each vertical column represents the results for the same serum obtained by the different penicillin preparations. In all the experiments the dosage was 20 mega units benzylpenicillin i.v.

It is apparent from the table that all the penicillin preparations elicited immediate wheal-and-flare reaction in the field sensitized with serum EJN. This serum had the highest titre value of penicilloyl-specific skin-sensitizing antibodies. Positive reactions were never observed with serum EMT.

As far as all serum samples are concerned, *positive reactions were elicited in 37 out of 63 cases with the commercial crystalline penicillins*. Identical results were found with the preparations from Novo, Glaxo, and Squibb, lot 8 A 898, while somewhat fewer and weaker reactions were observed with Purapen® and Squibb lot 8 E 619 (7 out of 16).

Two different dialysed and 3 sephadex-purified penicillin preparations, all specially made by Leo, differed distinctly from the other, above-mentioned commercial preparations. They elicited positive wheal-and-flare reaction only in serum EJN, and in addition once in serum EGC. In all, only 9 positive reactions occurred in 53 sensitized fields.

TABLE 3
Reactions Elicited in Human Recipients in Sites Sensitized by Penicilloyl-Specific Skin-Sensitizing Antibodies after Intravenous Injection of Various Benzylpenicillin Preparations, 20 Mega Units.

	Sera									
	EJN	ALJ	KU	EJ ₂	EMT	EGC	XHJ	EC		
Commercial Penicillin	1X47	+						—		
	1X47	+	+	+	—			—		
	8X36	+	+	+	—			—		
	16X17	+	+	—	—	+	+	—		
	16X17	+	+	+	—	+	—	—		
	7693/1	+	+	—	—	+	—	+		
	8A 898	+	+	+	—	+	—	—		
	8E 619	+	+	—	—	+	—	—		
Purapen®	H28/1	—	+	—	—	—	+	+		
Beecham										
Dialysed Penicillin	670928	—			—			—		
Leo	671012	—	—	—	—			—		
	671012	—	—	—	—			—		
Sephadex-purified Penicillin	671121	—	—	—	—			—		
	681022	—	—	—	—	—	—	—		
	680322	—	—	—	—	—	—	—		
	680322	—	—	—	—	—	—	—		
Leo	680322	+	—	—	—	+	—	—		
	680322	+	—	—	—	—	—	—		
Serum titre for penicilloyl-polylysine										
	1:10000	1:100	1:100	1:100	1:10	1:1	1:1	1:1	1:1	1:1

TABLE 4
*Reactions Elicited by Penicilloyl-Polylysine in Human Recipients in Penicilloyl-Specific
 Passive Transfer Tests after the Recipients had been Pre-treated with Various Benzylpenicillin
 Preparations, 20 Mega Units i.v.*

Sera									
	EJN	ALJ	KU	EJ ₂	EMT	EGC	XHJ	EC	KAH
Commercial Penicillin	1X47	—	—	—	—	—	—	—	—
	8X36 ¹	—	—	+	+	—	—	—	—
	16X17	—	—	—	—	—	—	—	—
	16X17	—	—	+	—	—	—	—	—
	7693/1	—	—	+	+	—	—	—	—
	8A 898	+	—	+	—	—	—	—	—
Purapen® Beecham	8E 619	+	+	+	+	—	—	—	—
	H 28/1	+	—	+	—	—	—	—	—
Dialysed Penicillin Leo	670928	—	—	—	—	—	—	—	—
	671012 ²	—	—	—	—	—	—	—	—
	671012 ²	+	—	—	—	—	—	—	—
Sephadex- purified Penicillin Leo	671121	+	+	+	+	+	—	—	+
	680322	+	+	+	+	+	—	—	+
Serum titre for penicilloyl-polylysine									
	1:1000	1:100	1:100	1:100	1:10	1:1	1:1	1:1	1:10

¹ Reactions not elicited with penicilloyl-polylysine until 18 hours after the injection of penicillin.
² Reactions elicited with penicilloyl-polylysine immediately after the injection of penicillin.

¹ Reactions not elicited with penicilloyl-polylysine until 18 hours after the injection of penicillin.

² Reactions elicited with penicilloyl-polylysine immediately after the injection of penicillin.

The positive reactions elicited with penicillin were totally inhibited by preceding injection of penicilloyl-polylysine 0.05 ml 50×10^{-5} M locally into the sensitized fields.

After the intravenous injection of benzylpenicillin, 20 mega units, 13 recipients were injected with penicilloyl-polylysine 0.05 ml 1×10^{-5} M intracutaneously into all sensitized fields (Table 4). As a rule, penicilloyl-polylysine was injected $2\frac{1}{2}$ – $3\frac{1}{2}$ hours after the injection of penicillin, when all positive wheal-and-flare reactions to the penicillin injection had subsided.

In recipients *pre-treated with commercial penicillin* Novo, Glaxo, and Squibb or Purapen® *a positive reaction with penicilloyl-polylysine could be elicited in 20 out of 65 fields*. In 45 cases, however, a reaction could no longer be elicited, indicating specific inhibition.

In these investigations a marked difference was found between the Leo penicillins purified by dialysis and those purified by sephadex. *The preparations purified by dialysis entailed in 17 out of 18 fields inhibition of subsequent wheal-and-flare reactions with penicilloyl-polylysine 1×10^{-5} M in the sensitized sites.*

Following sephadex-purified penicillin inhibition was observed in only 3 out of 16 cases.

In one case (Table 4, note 1) penicilloyl-polylysine was not injected locally into the sensitized fields until 18 hours after the infusion of penicillin. However, the inhibition of penicilloyl-specific reactions corresponded to that observed in the other experiments $2\frac{1}{2}$ – $3\frac{1}{2}$ hours after the injection of penicillin. In 2 cases (Table 4, note 2) penicilloyl-polylysine was injected immediately after the infusion of penicillin had been completed. In spite of the short latent period, complete inhibition of penicilloyl-specific reactions was observed.

Two recipients were sensitized, like the others, on the back with 9 serum samples all containing penicilloyl-specific skin-sensitizing antibodies. Thereafter treatment with sephadex-purified penicillin was started 20 mega units i.v. daily for 6 days. After the penicillin therapy had been completed, peni-

cilloyl-polylysine, 0.05 ml 1×10^{-5} M, was injected intracutaneously into all sensitized fields (Table 5). No positive reaction was observed in one of these two recipients (although strongly positive reactions were seen in penicillin-specific sera). In the other recipient weak reactions occurred in sera EJN and EJ 2.

TABLE 5

Reactions Elicited by Penicilloyl-Polylysine in Human Recipients in Penicilloyl-Specific Passive Transfer Tests after the Recipients had been Treated with Sephadex-purified Benzylpenicillin, 20 Mega Units i.v. Daily for 6 Days.

		<i>Sera</i>								
		EJN	ALJ	KU	EJ ₂	EMT	KÅH	XHJ	EC	EGC
Sephadex-purified	680322	—	—	—	—	—	—	—	—	—
Penicillin										
Leo	681022	+	—	—	+	—	—	—	—	—
Serum titre for penicilloyl-polylysine		1:1000	1:100	1:100	1:100	1:10	1:10	1:1	1:1	1:1

The results of studying the inhibitory influence of penicillin therapy upon penicilloate-specific passive transfer tests are shown in Table 6. 24 hours after sensitization with the 7 penicilloate-specific sera the recipient was treated with commercial benzylpenicillin, 20 mega units i.v. This elicited, in a few minutes, highly positive penicillin- and penicilloyl-specific reactions in all sera but HMP. 9 hours later, after all the positive reactions had subsided, benzyl-penicilloate, 0.05 ml 1.7×10^{-2} M, was injected intracutaneously into all sensitized fields. This elicited a faintly positive reaction to only a single serum (EGC). In the other sensitized fields there was total inhibition of the penicilloate-specific reactions.

TABLE 6

Reactions Elicited by Benzylpenicilloate in a Human Recipient in Penicilloate-Specific Passive Transfer Tests after the Recipient had been Pre-treated with Commercial Benzylpenicillin, 20 Mega Units i.v.

	Sera						
	MAS	EMW	KU	EKS	EGC	OEP	HMP
Commercial penicillin	+	+	+	+	+	+	—
Novo 16 × 17 20 mega units i.v.							
Benzyl penicilloate 1.7 × 10 ⁻² M 0.05 ml i.c.	—	—	—	—	+	—	—
Serum titre for penicilloate	1:100	1:100	1:10	1:10	1:10	1:1	1:1

DISCUSSION

Penicillin is the most commonly used antibiotic, and in relation to its benefits the side effects, predominantly of allergic nature, would seem on the face of it of little importance.

However, as mentioned in the introduction, the extensive use of penicillin entails sensitization of quite a large group of the population. In most cases there is a question of specificity for the penicilloyl radical.

Exposure to penicilloyl compounds in penicillin therapy may be studied, as previously mentioned (33), in non-allergic recipients pre-sensitized locally with penicilloyl-specific skin-sensitizing antibodies. A number of theoretical presuppositions appear to be fulfilled in using such an experimental model.

- 1) Passive transfer tests afford reliable and comparable results from recipient to recipient (48, 34).
- 2) Penicillin therapy does not affect the elicitation of passive transfer tests in a non-specific way (33).
- 3) Cross allergy or mutual inhibition of the elicitation of positive reactions between the 3 antigens: benzylpenicil-

lin, benzylpenicilloate, and the penicilloyl radical do not occur in passive transfer tests (35).

- 4) Passive transfer tests may be elicited repeatedly in the same site by successive injections of the same antigen in ever increasing doses. However, the reactions gradually become weaker (8). In successive injections of an antigen in the same quantities—or decreasing quantities—into a sensitized field a positive reaction is elicited only by the first injection (35).
- 5) Passive transfer tests to human skin may be elicited in a practically unchanged strength by injection of antigen even 5–10 days after the sensitization has been performed (15, 33).
- 6) Within the selected experimental conditions there is, after a positive reaction has been elicited, no visible dissociation between antigen and antibody in the sensitized field (33).
- 7) The quantities of antigen, in particular of the penicilloyl radical, required to elicit passive transfer tests on human skin, are of the same magnitude as the quantities which may elicit positive cutaneous reaction in penicillin allergies (35).

The results listed in Table 3 show that exposure to penicilloyl compounds occurred after treatment with all the studied penicillin preparations, if 20 mega units were injected intravenously. All the penicillin preparations elicited a penicilloyl-specific reaction in serum E.J.N. The exposure to penicilloyl compounds was greatest after treatment with commercial penicillin whereupon positive reactions were elicited in 37 out of 63 sensitized sites. Significantly fewer reactions were elicited by specially made purified penicillin preparations, *i.e.* 9 positive reactions in 53 sensitized fields. Using the χ^2 test for independency with Yate's correction: $\chi^2 = 19.3$, with $f = 1$ $P < 0.1$ per cent.

In the experiments the positive, penicilloyl-specific reactions were observed in the sensitized fields during or immediately

after the infusions of penicillin. The specificity appears to be definite, as the reactions were totally inhibited by preceding local injection of penicilloyl-polylysine. In one serum, KÅH, a positive delayed reaction was observed after infusion of penicillin. This reaction was not penicillin-, penicilloyl-, or penicilloate-specific.

The results given in Table 4 confirm that treatment with commercial penicillin preparations entails exposure to penicilloyl compounds. Positive reactions could subsequently be elicited in only 20 out of 65 sensitized fields by penicilloyl-polylysine. Unlike the results of the experiments in Table 3, the dialysed Leo preparations now proved no better, rather worse, than the commercial ones. Thus, reaction was elicited with penicilloyl-polylysine in only 1 of 18 sensitized fields following treatment of the recipient with dialysed penicillin. The difference between commercial and dialysed penicillin is, however, not statistically significant. In the χ^2 test $\chi^2 = 3.5$, with $f = 1$ 5 per cent $< P < 10$ per cent.

Sephadex-purified penicillin preparations were still significantly better than the commercial preparations in the experiments shown in Table 4. Following infusion of sephadex-purified penicillin, 20 mega units i.v., to the recipients reaction could still be elicited in 13 out of 16 sensitized fields. In the χ^2 test $\chi^2 = 11.5$ with $f = 1$ $P < 0.1$ per cent.

The inhibition of penicilloyl activity in the sensitized field appeared almost instantaneously after penicillin therapy (Table 4, note 2), and apparently it did not increase appreciably within the subsequent 18 hours (Table 4, note 1).

As previously assumed (33), the elicitation of the positive penicilloyl-specific reactions in the experiments listed in Table 3 must be attributed mainly to the occurrence of high-molecular penicilloyl compounds in the commercial penicillin preparations. Thus, far fewer positive reactions were observed following dialysed than commercial penicillin. The inhibition of the reactions in the penicilloyl-specific sera following penicillin therapy (Table 4) may presumably be due to high- as well as low-molecular penicilloyl compounds. Accordingly,

least exposure to penicilloyl compounds was observed after sephadex-purified penicillin.

Exposure to penicilloyl compounds in penicillin therapy, thus, may be reduced, but cannot be completely avoided by using sephadex-purified penicillin as used in the present experiments. A positive penicilloyl-specific reaction was elicited in all experiments in serum EJN. Furthermore, 6 days' treatment with sephadex-purified penicillin, 20 mega units daily, entailed inhibition of penicilloyl activity in 7 and 9 out of the 9 sensitized fields (Table 5). No definite signs of penicilloyl formation *in vivo* were observed in any of the experiments.

The results of the studies indicate that penicillin therapy entails only slight exposure to penicilloyl compounds and that this exposure is due chiefly to penicilloyl contamination of the commercial penicillin preparations. This may explain the varying frequency of penicillin allergy observed in patients treated with penicillin, and the dose dependence of such reactions as mentioned in the introduction. It explains also the fact that patients with a positive cutaneous reaction to penicilloyl-polylysine usually tolerate penicillin therapy. Presumably, they are not sufficiently exposed to penicilloyl compounds for an allergic reaction to be elicited. Remarkably the highest incidence of allergic reactions observed during penicillin therapy (Table 2) is of the same magnitude as the frequency of positive cutaneous reaction to the penicilloyl radical in randomly selected persons.

Exposure to penicilloate in penicillin therapy is more pronounced (cf. Table 6). The exposure does not seem to occur immediately after the infusion of penicillin, as positive penicilloate-specific reactions were not elicited by local injection of penicillin (35). The present study indicates, however, that penicilloate is formed during the hours subsequent to the injection of penicillin by gradual hydrolysis of the penicillin.

On the basis of the studies it must be concluded that controlled treatment series should be administered to a large number of patients using different penicillin preparations: commercial, dialysed, and sephadex-purified. Thereby it would

be possible to confirm the results submitted, viz. that sensitization to the penicilloyl radical and penicilloyl-specific allergic reactions in penicillin therapy may be reduced by using highly purified penicillin preparations. Penicillin- and penicilloate-specific reactions can probably not be reduced by purified penicillin.

SUMMARY

The penicilloyl radical is of the utmost importance in penicillin allergy, being in most cases the active antigen. Other antigens are represented by penicillin itself and penicilloate. Recent investigations have shown that commercial crystalline penicillin is regularly contaminated with penicilloyl, partly in the form of penicilloylated protein compounds and partly in the form of low-molecular polymeric penicilloyl compounds.

Exposure to the penicilloyl radical during treatment with various penicillin preparations was studied in human recipients non-allergic to penicillin. Prior to the penicillin therapy, the recipients were passively sensitized in several fields by intracutaneous injection of sera from penicillin allergies containing penicilloyl-specific skin-sensitizing antibodies. During penicillin therapy the exposure of the recipients to penicilloyl compounds could be studied locally in the sensitized sites. In part penicilloyl-specific positive wheal-and-flare reactions occurred in the sensitized sites and in part there occurred specific inhibition of later elicitation of reaction by penicilloyl-polylysine.

The studies show that parenteral penicillin therapy, by all the studied benzylpenicillin preparations, commercial as well as specially made, entails exposure to penicilloyl compounds. Furthermore, that this exposure to penicilloyl compounds may be significantly reduced by using sephadex-purified benzylpenicillin.

The quantities of penicilloyl radical to which a patient is exposed as contamination to commercial penicillin seem appreciable in relation to the quantities of penicilloyl compounds which perhaps form *in vivo*.

By using highly purified penicillin instead of the conven-

tional commercial preparations for human penicillin therapy it is presumably possible to reduce the frequency of sensitization to the penicilloyl radical as well as the incidence of penicilloyl-specific allergic reactions.

Exposure to penicilloate in penicillin therapy was assessed in the same way in penicillin therapy to a human recipient pre-sensitized by penicilloate-specific skin-sensitizing antibodies. The study indicated that during penicillin therapy there is a gradual, but considerable exposure to penicilloate which may elicit penicilloate-specific allergic reactions.

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