

Prediction of penicillin allergy by immunological tests

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This paper serves to evaluate objective immunological tests to predict penicillin allergy. Prospective immediate skin and serum tests were performed on a total of 218 patients with past histories of penicillin allergy who had major medical indication for penicillin and who were then treated with penicillin depending upon the test results. Skin tests were found to be valuable predictive tests for immediate (including anaphylactic) and accelerated urticarial allergic reactions to penicillin. The clinical value of these tests is discussed, as well as the possible meaning of the data obtained with regard to the immune mechanisms of various allergic reactions to penicillin.

Allergic reactions to penicillin occur in 2 to 5 per cent of patient courses of penicillin. It has been estimated that more than 1,000 anaphylactic deaths to penicillin have occurred in the United States alone.¹ We would estimate that anaphylactic reactions to penicillin occur with a frequency of about 1 to 5 per 10,000 patient courses of penicillin. The value of objective predictive tests for penicillin allergy† is twofold: to screen out the potential severe allergic reactor to penicillin (particularly the anaphylactic reactor) and to enable the physician to use these valuable drugs in patients who, though they have past histories of penicillin allergy, could presently tolerate penicillin therapy without allergic reaction.

A long-term prospective study has been going on in our laboratory to determine the predictive value of immediate skin tests and hemagglutination tests for penicillin allergy. The skin tests with benzylpenicilloyl-polylysine (BPL) and the minor determinant mixture (MDM) detect skin-sensitizing antibodies (reagins)

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†We refer mainly to benzylpenicillin, but these studies are largely applicable also to the semisynthetic penicillins and cephalosporin derivatives.

of benzylpenicilloyl specificity²⁻⁴ and the minor haptenic determinants' specificity,⁵⁻⁷ respectively. Hemagglutination assays of serum detect benzylpenicilloyl-specific antibodies of the IgM and IgG classes.⁸

These predictive tests were designed to detect mainly the potential immediate (including anaphylactic) and accelerated urticarial reactors to penicillin. These tests are based on the hypothesis that immediate and accelerated reactions to penicillin are mediated by reagins which are present prior to penicillin therapy and which are detectable by skin tests with both BPL and MDM.^{5, 9} Accordingly, patients with risk of such reactions would be among those with positive immediate skin tests to BPL and MDM. Also, according to this view, patients with negative skin tests to BPL and the MDM should tolerate penicillin therapy without immediate or accelerated urticarial reaction despite a past history of penicillin allergy.

The experimental approach used has been to study the patients with reasonable past histories of penicillin allergy who presently had serious indication for penicillin therapy. Skin tests and hemagglutination tests were done prospectively, and patients were then treated with penicillin. The results obtained in the first 128 patients studied^{10, 11} bore out the hypothesis tested.

The present paper reports⁴ our results on 90 additional patients.* This paper also summarizes the results of our total experience with 218 consecutive patients with past histories of penicillin allergy who were tested prior to penicillin treatment. The results indicate that negative immediate skin tests to BPL and the MDM virtually exclude the possibility of an immediate (and anaphylactic) allergic reaction to penicillin and markedly reduce the probability of an accelerated urticarial reaction to penicillin. By contrast, a positive skin test to BPL or to the MDM indicates a very high probability of an immediate or accelerated allergic reaction to penicillin, especially when penicillin is given to such patients in high dosage. The usefulness of hemagglutination tests is discussed. Although large scale studies at several centers will be required to evaluate the limits of reliability of these tests, they appear now to be of considerable clinical usefulness to predict penicillin allergy.

MATERIALS AND METHODS

Patients

The 90 patients from Bellevue and University Hospitals were of both sexes and from 6 to 79 years old. All had infectious diseases; most were acutely ill with fever; some were also chronically ill. None had lymphoproliferative diseases. All had reasonably acceptable histories of penicillin allergy. Histories could not be medically documented in the majority of cases. However, these were typical clinical situations in which the patients gave a reasonably acceptable past history of penicillin allergy, and one could not rule out the possibility that the patient may be presently clinically allergic to penicillin. The histories

*These data were presented in abstract at the Twenty-fifth Annual Meeting of the American Academy of Allergy, Feb., 1968.²⁷ The abstract reports one skin test-negative patient who developed immediate urticaria to penicillin. This patient (R. S., Table III) was subsequently shown to have in the sera reagins reactive with sodium benzylpenicilloate (see text).

obtained where as follows: Of the skin test-negative patients, 13 per cent had immediate reaction, 15 per cent accelerated urticaria, 53 per cent late urticaria or exanthema, 19 per cent did not remember manifestations. The alleged reaction had occurred less than 3 months prior to testing in 20 per cent, 3 to 12 months prior in 15 per cent, 1 to 3 years in 19 per cent, and more than 3 years in 46 per cent. Histories of skin test-positive patients are given in Table III. Here too, about 20 per cent did not recall the manifestations of the alleged reaction (questionable allergic history), and in 40 per cent the reaction had occurred more than 3 years prior to testing.

Skin test materials

Benzylpenicilloyl-polylysine (BPL) was BPO_6Lys_7 and used at a concentration of $1 \times 10^{-6}\text{M}$ of the conjugate.⁴ The minor determinant mixture (MDM)

Table I. *Relation of prospective skin tests to the occurrence of immediate and accelerated allergic reactions to penicillin (recent experience)*

Number of patients with history of penicillin allergy	Skin tests		Immediate or accelerated allergic reaction to penicillin treatment*
	BPL	MDM	
77	Neg.	Neg.	One had mild accelerated urticaria† (Patient F. R., Table IV)
1	Dermatographia		Not treated with penicillin†
4	Pos.	Neg.	One of two treated with penicillin developed accelerated urticarial reaction, the second developed late urticaria†
3	Neg.	Pos.	One treated with penicillin developed accelerated flush reaction, a second developed an immediate urticarial reaction†
5	Pos.	Pos.	Not treated with penicillin†

*In skin test-positive patients, penicillin therapy started by gradual administration. In skin test-negative patients, penicillin therapy initiated without attempt at desensitization.

†Four of the 77 skin test-negative patients developed late exanthematic reactions, one patient developed granulocytopenia.

‡The others were treated with other antibiotics.

Table II. *Relation of immediate skin tests to the occurrence of immediate and accelerated allergic reactions to penicillin (total experience)**

Number of patients with history of penicillin allergy	Skin tests		Immediate or accelerated reaction to penicillin therapy
	BPL	MDM	
185	Neg.	Neg.	1 had mild accelerated urticarial reaction†
1	Dermatographia		Not treated with penicillins
9	Pos.	Neg.	Not treated with penicillins
4	Neg.	Pos.	Not treated with penicillins
8	Pos.	Pos.	Not treated with penicillins
8	Pos.	Neg.	5 of 8 had accelerated urticarial reactions
3	Neg.	Pos.	2 had accelerated reactions (urticarial or diffuse flush); 1 had an immediate urticarial reaction

*Including data in Voss et al.¹⁰ and Levine et al.¹¹

†Six of the 185 developed exanthematic reactions.

contained crystalline benzylpenicillin, $2 \times 10^{-2}M$; sodium benzylpenicilloate, $1 \times 10^{-2}M$; benzylpenicilloate, $1 \times 10^{-2}M$; and sodium- α -benzylpenicilloyl-amine, $1 \times 10^{-2}M$. The 4 chemicals were dissolved in 0.07M monosodium phosphate adjusted to pH 7.5 with 0.3M NaOH. First, penilloic acid was dissolved, then penicilloate (addition of 0.3N NaOH was necessary to maintain pH 7.5), and then the other 2.

Further details of preparation, sterilization, and storage are given elsewhere.^{4-7, 9-11} Penicilloyl-polylysines homologous to BPL were prepared from methicillin, oxacillin, and ampicillin by methods identical to those used for BPL.⁴ Minor determinant mixtures were also prepared from these semisynthetics. They contained the semisynthetic penicillin, the alkaline hydrolysis product of the penicillin (presumably the penicilloate), and the decarboxylated

Table III. *Clinical and immunological findings in skin test-positive patients*

Patient	Age (years) and sex	Personal history of atopy	Indication for penicillin therapy	History of penicillin allergy	
				Type of reaction	Time since reaction
M. D.	37, M	Yes	Late latent syphilis	Questionable reaction	6 years
N. L.	25, M	No	Fever of undetermined origin (F.U.O.), possible SBE	Late urticarial reaction	4 years
R. M.	54, M	No	Bacterial pneumonia	Accelerated urticarial reaction	2 days
G. B.	54, M	No	F.U.O., possible bacterial arthritis	Late urticarial reaction	1 year
M. K.	68, M	Yes	Diabetic gangrene	Anaphylaxis to ampicillin	2 days
T. J.	34, F	Yes	Tubo-ovarian abscess	Exanthematic reaction to ampicillin	1 week
M. N.	46, F	Yes	SBE	Late urticarial reaction	3 years
W. N.	60, M	No	Sepsis	Exanthematic reaction	1 week
P. P.	69, M	No	F.U.O., possible SBE	Anaphylaxis	1 week
M. L.	50, M	No	Actinomycosis	Late urticarial reaction	3 days
H. C.	68, M	Yes	SBE	Accelerated reaction	2 years
R. S.	74, F	No	SBE	Questionable reaction	10 years

*Titers before and after treatment of the serum with 0.1M mercaptoethanol.

†Not treated with penicillin, semisynthetic penicillins, or cephalosporins. Other antimicrobial

‡Penicillin initiated by "desensitization" schedule.

§By passive transfer test, see text.

product of the latter (presumably the penicilloate), at the same concentration as in the MDM.

Skin test methods

Patients were skin tested with BPL, MDM, and a diluent control. If the patient was to receive semisynthetic penicillin, he was tested with the penicilloyl-polylysine and the MDM prepared from the semisynthetic, as well as with BPL and the MDM. Scratch tests were done first, and intradermal tests were done only if scratch tests were negative. Intradermal tests were done in duplicate with 0.005 ml. of the reagent (i.e., a minimally detectable bleb was raised). Tests were read in 15 minutes. Negative tests were 1 to 3 mm. blebs. Positive tests were sharply outlined wheals of 4 to 20 mm. in diameter with

<i>HA titer*</i>	<i>Skin tests</i>		<i>Allergic reaction to penicillin</i>
	<i>BPL</i>	<i>MDM</i>	
1,300/80	2+	0	Not treated with penicillin†
200/128	2+	2+	Not treated with penicillin†
800/400	0	2+	Not treated with penicillin†
400/400	1+	0	4.8 million units penicillin per day for 19 days, eosinophilia on eighth day, urticaria on tenth day‡
1,600/1,600	4+	3+	Not treated with penicillin†
64,000/64,000	3+	0	Not treated with penicillin†
100/100	2+	0	20 million units penicillin per day for 6 weeks, accelerated urticaria (12 hours), exanthematic reaction (48 hours), eosinophilia, Coombs positivity late‡
8,000/4,000	3+	3+	Not treated with penicillin†
32,000/32,000	2+	4+	Not treated with penicillin†
800/160	2+	2+	Not treated with penicillin†
64/0	0	2+	80 million units per day penicillin for 8 weeks, diffuse flush and wheezing during desensiti- zation (8 hours)‡
32/0	0	2+§	40 million units penicillin per day for 5 days (patient died), immediate diffuse urticaria 5 minutes after start of penicillin, exanthematic reaction, third day (see text)

drugs were used successfully.

surrounding flares. Reagents were nonirritating in nonsensitive control patients.

Other tests

Passive transfer tests were carried out by the classical Prausnitz-Küstner method as previously described.⁵ Hemagglutination assays were carried out as described previously.⁸

RESULTS

Frequency of positive skin tests

Twelve of the 90 patients (13 per cent) with past histories of penicillin allergy gave positive skin tests to BPL, MDM, or both (Table I). One patient gave equally intense reactions to the diluent control (he also displayed dermatographia, i.e., whealing to skin scratch) and could not be clearly evaluated by skin test. Our total experience to date, which includes 128 patients previously reported,^{10, 11} is shown in Table II. Thirty-two of 218 patients (15 per cent) with past histories of penicillin allergy had positive skin tests to BPL, MDM, or both. By contrast, among ward patients skin tested at random, positive immediate skin tests are detected in about 3 per cent.⁹

Specificity of skin tests

Table III shows that 4 of the 12 skin test-positive patients reacted only to BPL, 3 patients reacted only to the MDM, and 5 patients reacted to both the MDM and BPL. Of the 3 patients reacting only to the MDM, 2 reacted only to the sodium benzylpenicilloate constituent of the MDM and not to crystalline benzylpenicillin itself. A similar breakdown of specificity of skin tests was observed among the previously studied series of patients.¹¹

Three patients with positive skin tests to BPL were tested also with the ampicillin and oxacillin homologues of penicilloyl-polylysine (at 10^{-6} M). All 3 cross-reacted with almost equal intensity to the ampicillin homologue and to a lesser extent to the oxacillin homologue.

Relation of skin test positivity to past history of allergy

Of the 12 skin test-positive patients (Table III), 50 per cent had a history of a penicillin-allergic reaction one week or less prior to testing. This kind of history was found in only 10 per cent of the skin test-negative patients. There was no difference between skin test-positive and -negative patients with regard to the kind of allergic reaction to penicillin experienced in the past. The incidence of an atopic background was higher in the skin test-positive patient group (42 per cent) than in the skin test-negative group (12 per cent). The decreasing incidence of skin test positivity with time elapsed since the allergic reaction has been observed previously.¹²

Prediction of safety from immediate and accelerated urticarial reactions by negative skin test

The 77 skin test-negative patients in the presently studied group were all treated with penicillin. Fifty per cent were treated with benzylpenicillin, 33

per cent with a semisynthetic penicillin (ampicillin, oxacillin, methicillin, or combinations), 4 per cent with Keflin, and the remainder with a combination of benzylpenicillin and semisynthetic penicillins. The test material was administered intravenously in 50 per cent, intramuscularly in 25 per cent, and orally in 25 per cent. Dosage ranged from 400,000 to 40 million units of penicillin per day or the equivalent for semisynthetic penicillin. Therapy was continued for 5 days to 6 weeks. Patients were observed for allergic reactions during therapy and for up to 2 weeks after therapy had been discontinued. In all cases therapy was initiated at full dosage. No attempt at desensitization was made in these skin test-negative patients.

The results are given in Table I. None of the 77 developed an immediate allergic reaction. One patient (Patient F. R.) developed a mild accelerated urticarial reaction at 48 hours. Analysis of our total experience to date (Table II) reveals that none of 185 skin test-negative patients developed immediate allergic reactions to penicillin therapy, and only one patient, F. R., developed an accelerated allergic reaction. The clinical and immunological data in this exceptional case are presented in the Case report. These data would indicate that the accelerated urticarial reaction in Patient F. R. was mediated by reagins which were not present prior to penicillin therapy but were newly synthesized as part of an anamnestic immune response to penicillin.

Case report

Patient F. R. was a 65-year-old Puerto Rican man who presented with a clinical picture of subacute bacterial endocarditis (SBE) with multiple blood cultures positive for a penicillin-sensitive *Streptococcus viridans*. He gave a history of an accelerated urticarial reaction to penicillin therapy 10 years previously. He had not received penicillin therapy since. Skin tests with BPL and the MDM were negative, and the patient was started on penicillin therapy, 20 million units per day, intravenously. No attempt at desensitization was made. The patient was symptomatic for the first 48 hours of penicillin therapy. At that time, he noticed pruritis of his left arm, and 4 discrete urticarial lesions could be observed there. Penicillin therapy was continued; the urticaria faded spontaneously in a few hours. The patient was then asymptomatic until the sixth day of therapy, when he developed the 2 discrete hives on the left shoulder which faded spontaneously in 2 hours. On the eleventh day of penicillin therapy, he developed diffuse urticaria lasting for about 4 hours. Subsequently, he was asymptomatic for the remainder of his 6 week course of penicillin therapy. SBE was cured and has not recurred in the past 18 months of observation.

Table IV. Immunological findings in Patient F. R.

Days after initiation of penicillin therapy	Direct skin test		Passive transfer tests*		HA titer†
	BPL	MDM	BPL	MDM	
0 (1 hour before start of therapy)	Neg.	Neg.	Neg.	Neg.	32/0
2	Neg.	Neg.	1+	1+	32/0
5	Neg.	Neg.	ND‡	ND‡	ND‡
12	4+	2+	4+	4+	16,000/16,000
15	4+	3+	4+	4+	16,000/16,000
31	4+	3+	ND‡	ND‡	16,000/16,000

*0.1 ml. of serum injected intradermally into the deltoid area of recipients. Sensitized and control sites tested 48 hours later.

†Titers before and after treatment of the serum with 0.1M mercaptoethanol.

‡ND = not done.

The immunological findings are shown in Table IV. Reagins were not detectable by direct skin test or passive transfer test just prior to the initiation of penicillin therapy. At 48 hours, when the patient developed a mild accelerated urticarial reaction, reagins were detectable in the serum by passive transfer but were not detectable by direct skin test. Between the second and twelfth days, there was a rise in reagin titer, direct skin tests became positive, and BPO-specific IgG antibodies in high titer appeared.

These findings are consistent with the hypothesis that the accelerated urticarial reaction was mediated by reagins^{5, 9} and suggest that in this exceptional case the reagins were newly synthesized as part of an anamnestic immune response to penicillin.

Prediction of immediate and accelerated urticarial reactions by positive skin tests

Four of the 12 skin test-positive patients were treated with penicillin because of a serious indication for penicillin therapy (Table III). In 3 patients (Patients G. B., M. N. and H. C.) the penicillin therapy was initiated with gradually increasing doses under premedication with Benadryl (i.e., the so-called "desensitization" program). Two of the 3 developed accelerated allergic reactions to penicillin. Patient M. N. developed diffuse urticaria at 12 hours, and Patient H. C. developed a diffuse flush reaction with wheezing occurring at 8 hours after initiation of the penicillin in gradually increasing dosage. In both of these patients the reactions disappeared as antihistamine therapy was continued, and a complete course of penicillin was given with successful outcome. Patient G. B. developed late eosinophilia and urticaria. In the fourth patient, R. S.,* intravenous penicillin therapy, 40 million units per day, was initiated in full dosage without an attempt at desensitization. She developed diffuse urticaria 5 minutes after initiation of therapy. Therapy was discontinued, and urticaria disappeared spontaneously. Penicillin therapy, 40 million units per day, was restarted on the following day by gradual administration under Benadryl coverage. There was no further urticaria, but the patient developed a mild morbilliform eruption on the third day. She died from bacterial endocarditis (presumed cerebral embolization) 5 days later, however.

Analysis of our total experience to date (Table II) shows that of 10 skin test-positive patient treated by gradual initiation of penicillin therapy, 7 had accelerated reactions. The eleventh skin test-positive patient, whose treatment was initiated with full-dosage penicillin, had an immediate urticarial reaction. This patient reacted to the MDM and, more specifically, to the sodium benzylpenicilloate constituent of the MDM.

Prediction of exanthematic and late allergic reactions by skin tests

Four of the 77 skin test-negative patients developed exanthematic reactions associated with penicillin therapy. One patient developed this kind of reaction

*She had been considered to be a skin test-negative patient, as an MDM preparation lacking sodium benzylpenicilloate was erroneously used for testing. Subsequently it was found that the serum contained reagins reactive with only sodium benzylpenicilloate by passive transfer test, i.e., the skin test would have been positive to an MDM preparation containing that degradation product.

after 18 hours of high-dosage penicillin and oxacillin, another after 12 hours of high-dosage ampicillin, a third after 18 days of high-dosage penicillin therapy, and the fourth after 7 days of high-dosage penicillin therapy. In 2 patients, penicillin therapy could be continued with improvement or disappearance of the rash. In the third patient, tetracycline could be substituted, and the fourth had completed the course of therapy.

Of our total experience to date, 6 of 185 skin test-negative patients (3 per cent) developed exanthematic reactions associated with penicillin (or semi-synthetic penicillin) therapy. These reactions are not mediated by reagents⁹ and accordingly would not necessarily be expected to be predictable by skin tests (see Discussion). However, of 11 skin test-positive patients treated with penicillin, 3 (27 per cent) developed exanthematic reactions which followed the accelerated or immediate allergic reactions. These include Patients R. S. and M. N. (Table III) of the present study. Thus the incidence of exanthematic eruptions to penicillin therapy appears to be higher among the skin test-positive patients. This is likely to be due to an unusual immune response associated with the reagin response rather than to the reagents per se.*

One of the 185 skin test-negative patients developed local allergic inflammatory reactions to intramuscular injections of penicillin, which started on the eighteenth day of penicillin therapy. This was associated with the development of reagins and high titers of IgG antibody at that time. Serum drawn just prior to penicillin therapy showed a moderate titer of IgG antibodies (640/640).†

One patient of the 185 skin test-negative patients developed agranulocytosis (60 granulocytes per cubic millimeter) after tolerating 2 months of penicillin therapy (30 to 60 million units per day for osteomyelitis) without adverse reaction. The granulocyte count increased to normal between 2 and 5 days after penicillin was discontinued, and the patient recovered. The patient developed very high titers of BPO-specific IgM antibodies (12,000/800)† at the time of the granulocytopenic reaction.

Predictive value of the prospective presence of IgG and IgM antibodies

All 77 skin test-negative patients (and the one patient with dermatographia) had BPO-specific serum IgM antibodies in titers of 1:4 to 1:800; the median titer was 1:32. Twenty-two per cent had IgG antibodies, in addition, in titers of 1:32 to 1:1,600. Patient F. R. who developed an accelerated urticarial reaction to penicillin had only IgM antibody at a titer of 1:32, prior to initiation of penicillin therapy. The 2 patients who developed exanthematic eruptions within 24 hours of the initiation of penicillin therapy had prospective HA titers of 400/128 and 32/0, respectively. In our total experience with 185 skin test-negative patients, all had IgM antibodies and 21 per cent had IgG antibodies in addition. All but one (Patient F. R.) tolerated penicillin therapy without immediate

*It is possible that reagins (upon interaction with antigen) may enhance the development of some mobiliform reactions.

†Titers before and after treatment of the serum with 0.1M mercaptoethanol.

or accelerated urticarial reaction. In Patient F. R. the accelerated reaction appears to have been mediated by reagins (see Case report).

HA titers of the skin test-positive patients are shown in Table III. All the BPL-positive patients had BPO-specific antibodies, consistent with previous experience.⁹

DISCUSSION

The foregoing observations correlate the occurrence of allergic reactions to penicillin therapy with the results of prospective immunological testing of patients with histories of penicillin allergy. In general, the tests were found capable of detecting the potential immediate and accelerated allergic reactor to penicillin, as will be discussed later. First, two comments on the methods used: (1) Although the histories of penicillin allergy were not documented in most cases, these were reasonable histories and, accordingly, were typical clinical situations in which the physician has no way of knowing whether the patient will develop a severe allergic reaction on readministration of penicillin. (2) As to the immunological tests, BPO-specific IgG and IgM antibodies were assayed by a very sensitive passive hemagglutination method which can detect as little as 0.001 μ g per milliliter of rabbit BPO-specific antibody protein.⁹ Reagins (or skin-sensitizing antibodies) were detected by direct skin tests for wheal and flare reaction. Such tests are sensitive. They appear at present to be capable of detecting reagins in virtually all cases in which they are present. Thus, in general, the direct skin test has proved to be more sensitive than the classic Prausnitz-Küstner test in detecting reagins, with rare exceptions.¹³ The exceptions are situations in which reagin synthesis has just been initiated (i.e., the first 12 to 24 hours). Here reagins can be detected in the serum but not yet by immediate skin test, i.e., fixed to skin structures.¹³ Whether the skin test will prove to be more sensitive than newer serum tests for reagin, such as radioimmunodiffusion with anti-IgE serum,¹⁴ is not yet known.

Our experience in 218 consecutive patients permits several tentative conclusions: Negative immediate skin tests to both benzylpenicilloyl polylysine (BPL) and the minor determinant mixture (MDM) indicate that the patient will not have an immediate (or anaphylactic) allergic reaction to penicillin or that the risk of such a reaction is very low. Negative tests also indicate a very low risk of accelerated urticarial, asthmatic, or flush reactions to penicillin. An accelerated urticarial reaction did occur in one skin test-negative patient. The data presented indicate that this reaction was mediated by reagins whose synthesis was initiated after 48 hours of penicillin therapy as part of an anamnestic immune response to penicillin (Table IV). However, an accelerated reaction occurred in only one of 185 skin test-negative patients as compared with its occurrence in 8 out of 11 skin test-positive patients treated with penicillin.

Negative skin tests do not exclude the possibility of exanthematic reactions, Coombs positivity, or granulocytopenic reaction to penicillin (see Results), as these reactions are not mediated by reagins. However, at least the exanthematic reactions appear to occur considerably more frequently among skin test-positive

patients than among skin test-negative patients (see Results). Apparently the kind of immune response necessary for the mediation of exanthematic reactions is relatively commonly associated, or linked, with reagin production. Negative skin tests also do not exclude the possibility of a late urticarial reaction, as reagin production may begin some days or weeks after initiation of penicillin therapy. However, late urticarial reactions, too, were considerably more frequent among skin test-positive patients than among skin test-negative patients.

It is noted that skin testing with BPL alone, as has been done in earlier prospective studies,^{16, 17} or with BPL and penicillin is not sufficient to detect all potential immediate allergic reactors to penicillin. Thus, Patients H. C. and R. S. (Table III) gave positive skin tests only to the sodium benzylpenicilloate constituent of the MDM and gave negative skin tests to BPL and to penicillin. Both of these patients had impressive clinical allergic reactions to penicillin therapy: Patient H. C. had an accelerated flush reaction with wheezing, and Patient R. S. had an immediate diffuse urticarial reaction.

Positive skin tests indicate a high probability of an immediate or accelerated allergic reaction to penicillin. BPL-positive patients have a high probability of an accelerated urticarial reaction. Several factors appear to be important in determining whether a patient having BPO-specific reagins will develop such a reaction. Penicillin dosage is one factor. Thus, of the 8 BPL-positive patients treated with penicillin, all of the 4 patients treated with 20 to 40 million units per day developed accelerated reactions, whereas only 1 of 3 patients treated with 1.2 to 2.4 million units per day developed accelerated reactions (see Results). The eighth BPL-positive patient in this study was treated with oxacillin in high dosage and did not develop an allergic reaction. Oxacillin is only partially cross-reactive with benzylpenicillin in the penicilloyl system,¹⁸ which may account for this result. In studies by Rytel and associates¹⁶ and Brown and associates,¹⁷ only 5 to 15 per cent of BPL-positive patients treated with 1.2 million units of benzathine penicillin developed accelerated reactions.

A second factor appears to be the presence of BPO-specific IgG antibodies. These antibodies are present in virtually all patients with BPO-specific reagins (i.e., BPL-positive patients). The IgG antibodies can act as "blocking antibodies," i.e., antibodies which do not mediate urticaria-type allergic reactions themselves but which can compete with reagins for antigen (i.e., BPO-protein conjugates formed from penicillin *in vivo*) and thus prevent or modulate antigen-reagin binding and the subsequent specific release of histamine. Thus, in 4 of the 5 BPL-positive patients who developed accelerated reactions, urticaria began when the IgG antibody titer has been considerably reduced (from 75 to 97 per cent), and ceased spontaneously when IgG antibody titers rose over the initial values (as part of an anamnestic response to penicillin). Connell and associates¹⁹ have previously suggested for the ragweed system that IgG blocking antibodies may protect against constitutional reactions to injections of ragweed extract.

A third factor may be the binding affinity of the reagins. Binding affinity is basically a measure of the ability of antibody to bind antigen particularly when low concentrations of antigen limits binding. When low-affinity reagins

are present, a relatively high concentration of antigen would be required to initiate mediator release, and release would be more likely to occur slowly rather than explosively.

These 3 factors may explain the clinical observations, by Rytel and associates,¹⁶ Brown and associates,¹⁷ and those presented herein, that the great majority of BPL-positive patients do not develop immediate or anaphylactic reactions to penicillin. In the BPO-system, binding affinity of reagin may be generally relatively low, and/or the concentration or binding affinity of blocking antibodies may be relatively high. However, rare patients apparently can develop immediate urticarial reactions mediated by BPO-specific reagins.²⁰ In one case,²⁰ the circumstances accounting for this reaction appeared to be the combination of reagins of unusually high affinity and relatively low titers of blocking antibodies. In Patient M. N. (Table III), a BPL-positive patient who developed an accelerated reaction to penicillin, there was a decrease in IgG antibody titer preceding the urticarial reaction of only one tube. In this case, it may be that antibody affinity considerations were more important than antibody concentration considerations and that the time required for the buildup of BPO-antigen concentration (formed from penicillin *in vivo*) was a crucial determinant of the temporal aspects of this reaction.

In addition to the three factors already discussed, nonimmunological factors may also influence the occurrence of anaphylactic-type reactions in patients with BPO-specific reagins treated with penicillin. These may include tissue content of releasable mediators, blood vessel reactivity, histaminase activity in tissue, and other factors.

Minor determinant-specific skin test-positive patients appear to be those with the highest risk of immediate (including anaphylactic) allergic reaction to penicillin. This association has been repeatedly made in past studies.^{5, 9, 21} In the present study, Patient R. S. who had reagins reactive with benzylpenicilloate developed an immediate diffuse urticarial reaction to penicillin. The 2 other patients who also had reagins of that specificity developed diffuse flush reactions (in one case with wheezing) during desensitization to penicillin (under antihistaminic coverage) prior to initiation of full dosage. In another study,¹⁰ 2 patients with reagins reactive only with penicillin developed immediate allergic reactions (urticarial in one and asthma in the other) to 250 units of penicillin injected intradermally. In past studies, immediate and, particularly, anaphylactic reactions have been associated in the majority of cases with skin reactivity to penicillin itself,^{5, 9, 21} although retrospective associations of immediate reactions with reagins reactive with benzylpenicilloate are also seen.⁹

Two possible reasons for the observed preferential association of immediate allergic reactions to penicillin with minor determinant-specific reagins are: (1) Minor determinant-specific reagins may have unusually high binding affinities. (2) The concentrations of minor determinant-specific blocking antibodies may be relatively low. These possibilities seem attractive, as it is known that antibodies synthesized in response to small doses of antigen are generally of high affinity, at least for IgG antibodies in the rabbit.²² Also, relatively larger doses of antigen are needed to induce IgG antibody synthesis than are

needed for IgM antibody synthesis, at least for viral antigens in the guinea pig.²³ Accordingly, the idea that relatively smaller quantities of minor haptens than BPO haptens are formed from penicillin would be consistent with the hypothesis put forth previously. Examination of these possibilities experimentally will require the development of assay methods for IgG and IgM antibodies of minor determinant specificity, as well as methods for affinity measurements in these systems.

What is the value of the hemagglutinating (HA) IgM or IgG antibody titer in prediction of penicillin allergy? HA titers appear to have no value in predicting immediate reactions or accelerated urticarial reactions to penicillin in skin test-negative patients. All 185 skin test-negative patients had IgM antibodies, and 30 per cent had IgG antibodies in addition. They tolerated penicillin without such allergic reactions (except for Patient F. R., in whom reagin synthesis was initiated within 48 hours). Thus, in the penicillin system, non-reaginic antibodies appear incapable of mediating immediate or urticarial reactions, at least under the conditions we observed. IgG titers may be useful in judging the possibility of immediate reactions in BPL-positive patients. IgG titers may be of some value in predicting Coombs positivity induced by penicillin, and IgM titers may be of some value in predicting accelerated exanthematic eruptions and the rare cases of agranulocytosis associated with penicillin. Further, delayed skin tests to penicillin²⁴ may be of some predictive use.

In this study, there were no adverse reactions to the skin tests. By carrying out preliminary scratch tests and by performing intradermal tests only when scratches are negative (as was done here), the dose of antigen administered is kept as low as possible, and the probability of a constitutional reaction is decreased. In 2 reported cases of generalized reactions to skin tests with BPL,^{25, 26} excessively large doses of BPL were used, and preliminary scratch tests were not done. However, even with careful technique, there remains a very small but definite risk of constitutional reaction to skin tests in extremely sensitive patients. This kind of very small risk is present in any skin-testing procedure, but it is judged clinically acceptable, especially when it is weighed against the useful clinical information that such tests provide.

These objective predictive tests for penicillin allergy are thus convenient, safe, and apparently of a high degree of accuracy. The greatest value lies in the immediate skin tests with BPL and the MDM, which appear to have a very high order of accuracy in detecting the patient with risk of immediate (including anaphylactic) or accelerated urticarial reaction to penicillin. Large-scale studies at several centers are now needed to determine the limits of reliability of these tests. However they already appear of value in detecting those patients who, despite a history of penicillin allergy, can presently tolerate these drugs without anaphylactic reactions and in detecting the potential anaphylactic reactor to penicillin.

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