

From Rigshospitalet (The University Hospital), Medical Department P (Heads: J. Hess Thaysen, M.D. and Stig Jarnum, M.D.), Medical Out-patients Clinic, Division for Allergic Diseases (Head: Egon Bruun, M.D.) and the Finsen Institute, Medical Department (Head: Nis I. Nissen, M.D.), Copenhagen Denmark.

THE SEROLOGICAL DIAGNOSIS OF PENICILLIN ALLERGY

By

JENS PEDERSEN-BJERGAARD

INTRODUCTION

Since the first cases of an allergic reaction to penicillin were reported in 1943-44 (39, 53, 60, 77), problems relating to human penicillin allergy have attracted considerable interest. Diagnostic studies, clinical as well as serological, have been carried out on a large number of patients suspected of penicillin allergy.

Clinical studies using cutaneous tests with penicillin and penicillin derivatives have been performed by different techniques, but in most investigations they have proved unreliable for predicting manifest penicillin allergy. Therefore, supplementary provocation tests with penicillin have been tried. However, any test involves a risk, as serious allergic reaction may be elicited in very sensitive penicillin allergies even by cautious cutaneous tests. Furthermore, cutaneous tests and provocation tests may entail hyposensitization (68), perhaps also sensitization of patients to penicillin.

Serological studies have, therefore, attracted particular interest in diagnosing penicillin allergy, and various immunological tests have been tried, primarily determination of skin-sensitizing antibodies and agglutinating antibodies. In addition, a few more complex techniques have been employed. If a test is to be applicable clinically, it has to be extremely reli-

able. Treatment with penicillin on the basis of a "false negative" serological reaction may lead to systemic allergic reaction, perhaps of a fatal outcome.

Skin-sensitizing antibodies of the *Ig E* class play a pathogenetic role in man in various allergic diseases of the immediate type, spontaneous, hereditary (*e.g.* asthma and hay fever), as well as induced, non-hereditary (*e.g.* serum sickness) (10, 19). Thus, the hypersensitivity may be transmitted from an allergic subject to non-allergic recipients by the injection of serum containing skin-sensitizing antibodies. If the serum is injected intracutaneously a local sensitization may result as in the Prausnitz-Küstner's test (70), if serum is injected intravenously the recipient may acquire generalized passive sensitization (28).

The demonstration of skin-sensitizing antibodies in man is of practical diagnostic interest, a positive finding usually being tantamount to manifest allergy with a simultaneous positive intracutaneous reaction of the immediate type to the antigen in question (18, 72). Furthermore, titre values of skin-sensitizing antibodies in several allergic diseases, *e.g.* hay fever, are to some extent correlated to the clinical sensitivity (17).

In penicillin allergy investigations for skin-sensitizing antibodies by the Prausnitz-Küstner technique (70) have most often been performed on single cases, only a few of the publications dealing with series of patients (Table 1). Independent specificity occurs for at least 3 different chemical determinants represented by benzylpenicillin, penicilloyl-polylysine, and benzylpenicilloate (47, 65).

The incidence of demonstrable skin-sensitizing antibodies in alleged penicillin allergies varies with the reliability of the diagnosis, the clinical type of reaction, and the number of antigens used. With the 3 antigens mentioned skin-sensitizing antibodies have been demonstrated in 55 per cent (64) and 47 per cent (72) of penicillin allergies (Table 1). Penicillin specificity is most often found in cases of anaphylactic reactions, penicilloyl specificity in cases of urticaria.

Prediction of clinically manifest penicillin allergy has been

TABLE 1
Occurrence of Skin-Sensitizing Antibodies in the Serum of Patients with Alleged Penicillin Allergy.

Authors	Year	Clinical reactions	No. of pts. tested	Percentage with skin-sensitizing antibodies		
				Penicillin	Penicilloyl	None demonstrable
Criep	(20)	Urticaria	1	100		0
Callaway & Barefoot	(15)	Urticaria	5	0		100
Goldman et al.	(31)	Urticaria	7	0		100
Truitt	(92)	Serum sickness	2	100		0
Hopkins & Lawrence	(37)	Urticaria	4	0		100
Mayer et al.	(57)	Anaphylactic	5	80		20
Matheson & Elegant	(56)	Various	6	66		34
Linde	(51)	Anaphylactic	1	100		0
Maganzini	(55)	Anaphylactic	2	0		100
Käss & Ødegaard	(43)	Anaphylactic	1	100		0
Ljaljevic	(52)	Various	32	47		53
Reisman et al.	(74)	Various	19	63		37
Siegel	(86)	Anaphylactic	18	95		5
van Arsdel et al.	(6)	Various	8	50	50	25
Siegel & Levine	(87)	Anaphylactic	5	80	20	0
		Urticaria	2	0	100	0
Redmond & Levine	(72)	Various	40	20	30	53
Bierman & van Arsdel	(11)	Various	160*	6	9	90
Pedersen-Bjergaard	(64)	Various	53§	28	23	45

* = only patients with positive cutaneous reactions were studied.

§ = diagnosis confirmed by provocation test.

Blank = investigation not performed.

attempted by studies for skin-sensitizing antibodies. In a few investigations (74, 86, 87) it has seemed possible to rule out immediate reactions, in particular anaphylactic, by investigation for skin-sensitizing antibody using penicillin and penicilloyl-polylysine. But it is doubtful whether these findings can be confirmed and whether a demonstration of skin-sensitizing antibodies to penicillin derivatives is invariably tantamount to clinically manifest penicillin allergy.

The Prausnitz-Küstner technique, used in the reported studies, is unsuited for extensive clinical use, and attempts have been made to replace it by passive sensitization of monkey tissue. Sensitization of monkey skin (P.C.A. technique) by sera from penicillin allergies is possible (64), and skin-sensitizing antibodies of the Ig E class may be demonstrated, but the method is not sufficiently sensitive. Passive sensitization of monkey intestine (4) is more sensitive and may perhaps become clinically applicable in the future.

Agglutinating and precipitating antibodies may often be demonstrated in human allergy of the immediate type. Agglutinating antibodies may be 7S or 19S globulins (32) of the Ig G, Ig M, and perhaps Ig A class (36). Precipitating antibodies are of the Ig G type (99).

In man agglutinating antibody must be considered pathogenic, in association with antigen action, for immune haematological states, such as *e.g.* Sedormid purpura (1), Pyramidon agranulocytosis (59), and quinidine-induced haemolytic anaemia (26). In experimental animals, precipitating antibody may cause an Arthus reaction upon injection of antigen (41).

In human serum sickness, skin-sensitizing antibodies have been demonstrated, as well as precipitating and agglutinating antibodies with correlation between agglutinin titre and clinical parameters (2). Moreover, the histological findings are reminiscent of the Arthus reaction. It has been assumed, therefore, that agglutinating and precipitating as well as skin-sensitizing antibodies may play a pathogenetic role (9). Precipitating antibody is perhaps also operative in farmer's lung (40), baggassosis (78), and pigeon breeder's disease (73).

TABLE 2
Occurrence of Agglutinating Antibodies to Penicillin-Sensitized Erythrocytes in Persons with and without a History Suggesting Penicillin Allergy.

Authors	Year	Method	Patients with history of penicillin allergy		Patients without a history of penicillin allergy	
			No. of pts. tested	Percentage with positive react.	No. of pts. tested	Percentage with positive react.
Ley et al.	1958	pH 6.1	27	27	2000 rand. §	1.3
Ley et al.	1958	pH 6.1	20	45		
Epp	1959	B.D.B.	62 rec.	29	70	4
Heggie	1960	pH 6.1	49 prev.	4		
Harris & Vaughan	1961	pH 5.0	16	38	474	1
van Arsdel et al.	1963	pH 6.1	140	36	151	7
Schwartz & Vaughan	1963	pH 6.1	54 rec.	59	27 p.t.	33
			24 prev.	50	108 rand.	7
Budd et al.	1964	pH 8.6†	22 rec.	77	11 p.t.	45
			15 prev.	20	9 rand.	22
Thiel et al.	1964	pH 8.6†	114	65	68	9
de Weck	1964	pH 7.4* & pH 9.0*	107	pH 7.4 12 pH 9.0 52	72	pH 7.4 1.3 pH 9.0 2.8
de Weck & Blum	1965	pH 9.0*	94 rec.	56	176	6
			98 prev.	19		
Radermecker & Salmon	1966	pH 6.1	14 rec.	71	43 p.t.	16
			21 prev.	10	195 rand.	5
Bierman & van Arsdel	1969	pH 6.1	160	8		

§ = a few penicillin allergies included. * = agglutination reaction recorded only at a positive titre $\geq 1:4$. † = agglutination reaction recorded only at a positive titre $\geq 1:16$. B.D.B. = bis-diazotized benzidine. pH = pH at sensitization of erythrocytes. rec. = recent allergic reaction. prev. = allergic reaction a long time ago. p.t. = recent penicillin therapy. rand = randomly selected patients.

In atopic, hereditary allergic diseases agglutinating and precipitating antibodies are of more questionable importance. Thus, the titre values of ragweed-specific agglutinating antibodies in patients with untreated hay fever are not correlated to the clinical symptoms and signs. Furthermore, agglutinating antibodies to ragweed and cow's milk protein are frequently present in persons without clinical signs of pollen or milk allergy (3, 79).

Penicillin-specific precipitating antibodies are claimed to have been demonstrated in the serum of penicillin allergies (20, 61, 100). The precipitates, usually of a delayed occurrence are presumably non-specific, and it has not been possible to confirm the results (46, 58, 94).

Agglutinating antibodies to penicillin-sensitized erythrocytes were first demonstrated in man by *Ley et al.* 1958 (50). Antibody, occasionally in a high titre, was found in 1.3 per cent of randomly selected sera, in most cases from persons showing no evidence of clinical penicillin allergy. By the same technique an increased frequency of agglutinating antibody was later found in penicillin allergies and in patients who had recently completed courses of penicillin therapy (Table 2). The sensitivity of the agglutination reaction could be increased by sensitizing erythrocytes with penicillin at alkaline reaction and using normal serum-dextran mixture as diluent (44). Thereby, agglutinating antibody could be demonstrated in 97 per cent of all human sera tested.

Agglutinating antibody to penicillin-sensitized erythrocytes may be 7S or 19S globulin (29, 44, 82) of the *Ig G* or *Ig M* class (46). The specificity is predominantly, but not exclusively, directed at the penicilloyl radical in the D- α configuration (91) or more probably the α -diastereoisomeric configuration (45). The pathogenetic role of these commonly occurring, penicilloyl-specific agglutinating antibodies in human penicillin allergy has not yet been finally elucidated.

Immune-haemolytic anaemia may arise in the course of penicillin therapy, caused by a reaction of agglutinating antibody with erythrocytes sensitized to penicillin *in vivo* (7, 8, 16, 62,

69, 89). This reaction is not common, but may cause a considerable decrease in haemoglobin. The direct Coombs test is positive, and frequently there are other allergic manifestations, *e.g.* urticaria (89), erythema (69), or exanthema (16). Cross allergy between penicillin and cephalothin has been observed in penicillin-induced haemolytic anaemia (62), corresponding to what has been observed in studies on skin-sensitizing antibodies (63).

Arthus-like local reactions have been reported after injections of penicillin (14, 81), also in association with conditions suggesting serum sickness (25) or purpura and peripheral gangrene (23). Further, there have been reports of penicillin-induced leukopenia (21, 88) and thrombocytopenic purpura (54, 97). Agglutinating antibodies may have elicited these rare reactions, but unfortunately no relevant immunological investigations have been performed. Several authors have presumed that agglutinating antibody of the Ig M and Ig G type may be of pathogenetic significance in eliciting exanthemas and other common reactions to penicillin. This hypothesis has not yet been confirmed.

The "*basophil degranulation test*" was introduced by *Shelley & Juhlin* in 1961 (83) as being well-suited for the diagnosis of penicillin allergy. The technique was later modified (84, 85). The test must be interpreted as an *in vitro* equivalent of basopenia in certain allergic reactions (76), but it has been regarded with great scepticism (12, 27). It is technically difficult, and it is not known whether it bears a direct correlation to the occurrence of skin-sensitizing or agglutinating antibodies. In spite of promising results (38), it is hardly applicable, in its present form, in the clinical diagnosis of penicillin allergy owing to positive reactions in 15 per cent of non-allergics (85).

"*In vitro* blastogenic response of lymphocytes" to antigenic action has been employed in recent years in human penicillin allergy (24, 30, 33, 42, 80). Using morphological assessment of the blast transformation some authors have obtained a good dissociation between penicillin allergies and non-allergic

controls (24, 33, 42). However, spontaneous blast transformation has been observed in association with allergic reactions to penicillin (30), and in an assessment of the blast transformation by ^{14}C -thymidine only one positive result was found among 15 penicillin allergics (80).

There is reason to mention yet two other immunological methods which may possibly acquire decisive importance in the serological diagnosis of penicillin allergy:

The *radio-allergosorbent test*, developed by *Wide, Bennich & Johansson* in 1967 (98) enables a quantitative *in vitro* determination of Ig E with specificity for a given antigen.

The *Leucocyte migration test*, originally described by *Rich & Lewis* in 1932 (75) and modified by *Søborg & Bendixen* (90), affords a specific, quantitative *in vitro* determination of delayed-type hypersensitivity in man.

Unfortunately, results of these two tests in penicillin allergics have not yet been submitted.

OBJECT OF THE PRESENT STUDY

Investigation for agglutinating antibody to penicillin-sensitized erythrocytes was performed, using varied techniques and inhibition studies on sera from proven penicillin allergics and non-allergic control persons. Attempts were made to correlate the results to findings of skin-sensitizing antibodies in the same sera (64) and to clinical parameters (68) with a view to predicting manifest penicillin allergy on the basis of serological findings.

MATERIALS AND METHODS

Sera

Sera from 53 patients with manifest penicillin allergy (= *penicillin allergics*). The diagnosis had been confirmed in 49 by provocation tests with penicillin and in 4 by a characteristic history and either a positive immediate-type intracutaneous reaction to penicillin or demonstrable penicillin-specific, skin-sensitizing antibodies in the serum (64, 68).

Sera from 12 patients who tolerated penicillin in therapeutic doses in spite of a history suggesting penicillin allergy (= *patients with an alleged, but not confirmed history of penicillin allergy*).

Sera from 120 patients *without any history of penicillin allergy* described clinically as control group A in a previous paper (68).

All sera were removed and stored sterile at -20°C until the investigations could be carried out.

Reagents

- (a) Commercial crystalline benzylpenicillin as the sodium salt: Penicillin Leo® (Leo Pharmaceutical Products Trading Ltd., Copenhagen) or Penicillin Novo® (Novo Industri A/S, Copenhagen) stored at room temperature as dry sterile powder in rubber-capped vials of 10^6 , 2×10^6 and 10^7 units.
- (b) Alsever's solution: pH 6.1, per litre sodium citrate 8.0 g; sodium chloride 4.2 g; citric acid 0.55 g; glucose 20.5 g. The solution was prepared aseptically and stored at 4°C .
- (c) Phosphate-buffered saline: pH 7.2, sterile, isotonic, 0.01 M phosphate-buffered saline.
- (d) Coombs' anti-human globulin sera from rabbits: 1414 x, 1393 x and 1236 x, Blood Bank Laboratory, Statens Seruminstitut, Copenhagen.
- (e) 2-Mercaptoethanol Pure, (M 37 p), Koch Light Lab. Ltd., Colnbrook Bucks., England.
- (f) Penicilloyl-polylysine: "Cilligen"® PPL 1 and PPL 50 containing 1×10^{-5} and 5×10^{-4} M penicilloyl compounds (Sigma Chemical Company, St. Louis, Missouri, U.S.A.), stored at 4°C .
- (g) Benzylpenicilloate as the sodium salt, 1.7×10^{-4} M solution (Leo Pharmaceutical Products Trading Ltd., Copenhagen), described in a previous publication (64), stored at -20°C .
- (h) Evans Blue (T 1824) 0.5 per cent aqueous solution (U.S.P. Warner Chilcott).
- (i) Veronal buffer: pH 9.0, sterile, 0.1 M stored at 4°C .

Methods

(1) *Agglutination studies* were carried out by a modified Ley's technique (5, 67), incubating erythrocytes with penicillin in Alsever's solution pH 6.1.

Human blood, group O Rh neg., in a few cases O Rh pos., was stabilized immediately by equal parts of Alsever's solution. This suspension was thereafter incubated with half a volume of Alsever's solution with and without benzylpenicillin, 112 mg/ml, at 37°C for 75 min and left overnight at 4°C . Sensitized as well as non-sensitized erythrocytes were then washed 4 times, after centrifugation, with a large volume of phosphate-buffered saline. Erythrocyte suspension was prepared from 3.0 ml phosphate-buffered saline + 0.2 ml erythrocytes.

Test serum, standard serum (with known agglutinin titre), and normal serum (serum from the erythrocyte donor) were incubated in a water bath of 56°C for 30 min to inactivate complement. Serial twofold dilutions of the test serum and standard serum were prepared with aliquots of 0.1 ml normal serum as the diluent in 2 ml test tubes.

To each test tube with 0.1 ml serum dilution 0.01 ml erythrocyte suspension was added. The tubes were thoroughly shaken, and after being left at room temperature for 10 min centrifuged for 1-2 min at 1000 r.p.m. The tubes were then cautiously shaken by hand to resuspend the erythrocytes, and the agglutination reactions were read directly (Fig. 1).

Control experiments were done using

1. each test serum + non-sensitized erythrocytes from the same donor as the sensitized ones,
2. standard serum in dilutions + penicillin-sensitized erythrocytes from each erythrocyte donor,
3. normal serum + penicillin-sensitized erythrocytes from each erythrocyte donor,

in order to rule out false positive and false negative agglutination reactions.

All sera were investigated at least twice in satisfactory experiments. Fresh erythrocytes were prepared daily for the experiments.

(2) *Supplementary agglutination studies* were done incubating erythrocytes with penicillin in veronal buffer pH 9.0 as reported by *de Weck* (95) with minor modifications.

Human blood, group O Rh neg., in a few cases O Rh pos., was stabilized immediately by equal parts of Alsever's solution. Erythrocytes were washed 4 times with phosphate-buffered saline. Erythrocytes, 10 per cent suspension in phosphate-buffered saline, were incubated with equal volume phosphate-buffered saline with and without benzylpenicillin 50 mg/ml and half a volume Veronal buffer pH 9.0 at 37° for 1 hour. Sensitized as well as non-sensitized erythrocytes were then washed 4 times with a large volume of phosphate-buffered saline, and erythrocyte suspension prepared from 2.0 ml phosphate-buffered saline + 0.1 ml packed erythrocytes.

Sera were incubated at 56° C for 30 min to inactivate complement, and serial twofold dilutions of test serum were prepared with phosphate-buffered saline as diluent. To each test tube with 0.1 ml serum dilution 0.01 ml erythrocyte suspension was added, and agglutination was accomplished with test serum and normal serum as described above.

All sera were investigated at least twice in satisfactory experiments. Fresh erythrocytes were prepared daily for the experiments.

(3) *Agglutination studies using Coombs' anti-human globulin sera* were carried out introductory in accordance with item (1). After incubation of sensitized and non-sensitized erythrocytes with test serum for 10-20 min the erythrocytes were cautiously washed 4-5 times with—and re-suspended in—phosphate-buffered saline. Different Coombs' sera were added in varying dilution and quantity, and after 10 minutes' incubation with occasional shaking, cautious centrifugation was done. The tubes were then shaken by hand in order to re-suspend the erythrocytes, and the reactions were read directly.

(4) *Inhibition of agglutination reactions* was performed by incubation of test sera with benzylpenicillin in varying concentration or with 2-mercaptoethanol. After the inhibition, serial twofold dilutions of inhibited as well as non-inhibited test serum were prepared simultaneously, and agglutination reactions were carried out as described under item (1). The investigations were done at least twice in satisfactory experiments for each serum.

In inhibition studies with penicillin, the test serum was incubated for one hour at 20° C as a rule with equal parts of a benzylpenicillin solution containing 1, 10, 10², 10³, 10⁴ possibly 10⁵ and 10⁶ units per ml. In the inhibition studies with 2-mercaptoethanol 0.1 ml test serum was incubated for 2 hours at 20° C with 0.3 ml 0.133 M 2-mercaptoethanol as described by Levine *et al.* (46).

(5) *Passive transfer tests to monkey skin* were performed on 11 Macaca-irus (cynomolgus) monkeys weighing 2.8–5.7 kg, average 4.7 kg. It was endeavoured to elicit reactions by benzylpenicillin and penicilloyl-polylysine as reported previously (64).

(6) *Passive transfer to human skin* by the Prausnitz-Küstner technique (70) was performed at least twice with each serum on satisfactorily reacting recipients. It was endeavoured to elicit reactions by benzylpenicillin, penicilloyl-polylysine, and benzylpenicilloate as reported previously (64).

(7) *Provocation tests* were performed by intramuscular injection of benzylpenicillin 10⁶ units. After the injection the patients were carefully observed for one hour for immediate allergic reactions, and 7–14 days later they were again investigated for delayed allergic reactions.

RESULTS

Agglutinating antibodies to pII 6.1 penicillin-sensitized erythrocytes were recovered in the sera of 25 out of 53 penicillin allergics, in 5 out of 12 patients with alleged but not confirmed penicillin allergy, and in 8 out of 120 patients without a history of penicillin allergy (Table 3). Intramuscular provocation by benzylpenicillin 10⁶ units in the 8 patients with agglutinating antibody without a history of penicillin allergy did not in any case elicit demonstrable allergic reaction.

Agglutinating antibodies to pII 9.0 penicillin-sensitized erythrocytes were recovered in the sera of 25 out of 52 penicillin allergics, in 5 out of 11 patients with alleged but not confirmed penicillin allergy, and in 20 out of 120 patients without a history of penicillin allergy (Table 3). A positive reaction oc-

curred in 13 patients with a negative agglutination reaction to pH 6.1 sensitized erythrocytes, one penicillin allergic and 12 patients without a history of penicillin allergy. Out of the latter 12 patients 10 were provoked with benzylpenicillin 10^6 units intramuscularly without any complaints and one patient

AGGLUTINATION EXPERIMENT WITH PENICILLIN-SENSITIZED ERYTHROCYTES

TEST SERUM + PENICILLIN-SENSIT. ERYTHROC.

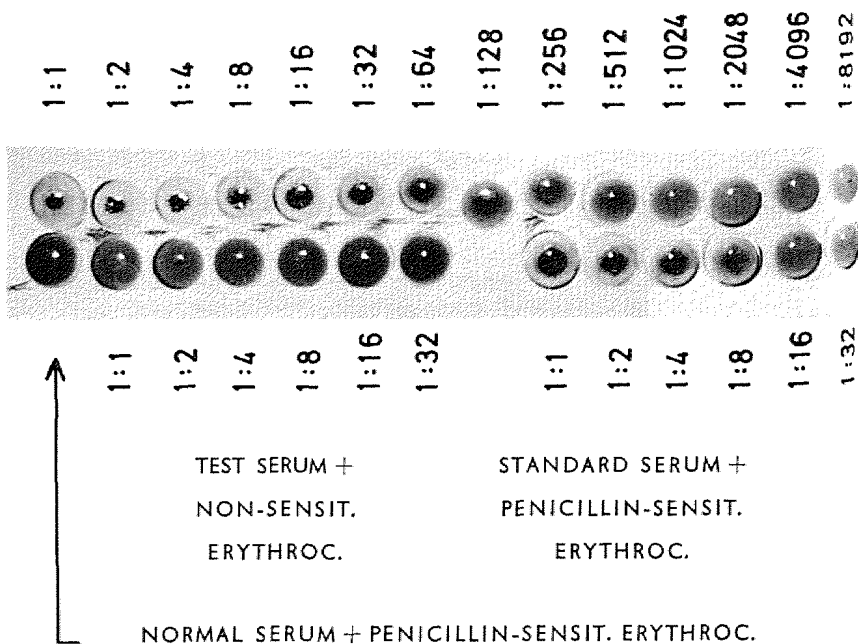


Fig. 1.

TABLE 3
Investigation for Agglutinating Antibodies to Penicillin-Sensitized Erythrocytes in 53 Penicillin Allergics, 12 Patients with Alleged but Not Confirmed Penicillin Allergy, and 120 Patients without a History of Penicillin Allergy.

<i>Incubation of Erythrocytes with Penicillin at pH 6.1</i>												
Neg.	Titre of agglutinating antibodies											
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
53 penicillin allergics	28	5	3	1	1	2	4	3	1	0	2	1
12 pts. with alleged but not confirmed penicillin allergy	7	2	0	1	0	0	0	1	1	0	0	0
120 pts. without a history of pen. all.	112	3(1*)	1	1(1*)	1	0	0	1(1*)	1	0	0	0
<i>Incubation of Erythrocytes with Penicillin at pH 9.0</i>												
Neg.	Titre of agglutinating antibodies											
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
52 penicillin allergics	27	2	6	2	2	5	4	1	0	1	0	1
11 pts. with alleged but not confirmed penicillin allergy	6	1	0	1	2	0	0	1	0	0	0	0
120 pts. without a history of pen. all.	100	5§	4	6(2*)	2(1*)	1	1	1	0	0	0	0

(*) number thereof with pos. cutaneous react. and skin-sensitizing antibodies to penicilloyl-polylysine.

§ 1 of these patients, when seen again, "remembered" previous penicillin allergy.

failed to attend for the provocation test. The last patient with agglutinating antibody in a titre of 1:1 to pH 9.0 sensitized erythrocytes and yet another patient with a doubtful reaction had denied previous penicillin allergy at the initial investigations. When these patients were seen again for a provocation test, both "now remembered" that many years ago they had had allergic reactions to penicillin, exanthema and exanthema with angioneurotic oedema.

The titre values of agglutinating antibody (mean value of several measurements) ranged in the different sera from 1:1 to 1:4096 (Table 3). The reactions were distinct (Fig. 1) and reproducible. Using erythrocytes sensitized at pH 6.1, thus, a total of 141 titre determinations were performed on 38 positive sera. For 28 sera the variations of the titre values were within ± 1 tube in the dilution series, for 9 within ± 2 tubes, and for only 1 serum within ± 3 tubes. Titre variations in excess of ± 1 tube were apparently due to a very few particularly high or low titre determinations, unrelated to the batch of erythrocytes or the experimental date.

Investigation by Coombs' anti-human globulin sera usually gave a positive reaction to sera from penicillin allergies, showing a positive direct agglutination reaction as described under items (1) and (2). No reproducible positive results were found in sera from penicillin allergies which had given a negative reaction in the direct agglutination tests.

Haemagglutination inhibition with penicillin was performed on all agglutinating sera except from patient MPA. Total inhibition was obtained for all sera at penicillin concentrations of between 5 and 5×10^5 units per ml serum. The relations between the agglutinin titre and the penicillin concentration needed for inhibition is given in Figs. 2 and 3 for the 37 sera. Sera from patients without manifest penicillin allergy and from penicillin allergies without demonstrable skin-sensitizing antibodies required a relatively higher concentration of penicillin for total inhibition than did sera from penicillin allergies with demonstrable skin-sensitizing antibodies.

Haemagglutination inhibition with 2-mercaptoethanol was

performed, because of simultaneous serum dilution, only on sera with agglutinin titres $\geq 1:8$ to pH 6.1 sensitized erythrocytes. Complete inhibition was obtained with certain sera from penicillin allergies (KÅH, OEP, OMJ, IV, and MHJ after hyposensitization with penicillin). Other sera were not inhibited (ASJ, EMT, EMW) or only partially, showing reduction of titre values within 2–5 tubes in the dilution series (EJN, HMP, EGC, and DJ). With a few sera (MOP, KU, EJ 2, and EC) the results varied from test to test. Sera from 3 patients without a history of penicillin allergy and 1 patient with alleged but not confirmed penicillin allergy were not or only slightly affected by 2-mercaptoethanol.

Passive transfer to monkey skin of sera from 12 patients with alleged but not confirmed penicillin allergy and 120 persons without a history of penicillin allergy did not in any case show a positive result upon challenge with benzylpenicillin or penicilloyl-polylysine. Owing to the shortage of monkeys, the study of the 120 sera could regrettably not be performed more than once on satisfactorily reacting monkeys.

Passive transfer to human skin of sera from 12 patients with alleged but not confirmed penicillin allergy showed a negative result upon challenge with benzylpenicillin, penicilloyl-polylysine, and benzylpenicilloate. In the sera from 3 patients with positive cutaneous reaction to penicilloyl-polylysine, but without a history of penicillin allergy, penicilloyl-specific skin-sensitizing antibodies in titre values of 1:1, 1:10, and 1:10 were demonstrated. Reactions to benzylpenicillin and benzylpenicilloate were negative. All 3 patients tolerated benzylpenicillin 10^6 units intramuscularly without complaints.

DISCUSSION

The demonstration of agglutinating antibody to pH 6.1 penicillin-sensitized erythrocytes in 47 per cent of 53 patients with definite penicillin allergy, in 42 per cent of 12 patients with alleged but not confirmed penicillin allergy, and in 6.5 per cent of 120 patients without a history of penicillin allergy

(Table 3) is in conformity with the results of several previous studies (Table 2). The incidence of agglutinating antibody was significantly higher in penicillin allergies than in persons without a history of previous penicillin allergy ($P < 0.001$, χ^2 test).

As might be expected, patients without a history of penicillin allergy had the highest frequency of agglutinating antibody after recently completed courses of penicillin (Table 4). In contradistinction, patients with definite penicillin allergy showed the same frequency of agglutinating antibody whether they had recently completed courses of penicillin therapy or been in contact with penicillin more than 3 months ago (Table 4). This result is not in keeping with some previous investigations (71, 96) (Table 2) but in accordance with another (82). The high incidence of agglutinating antibody in penicillin allergies was not due to a preponderance of recently penicillin-treated patients.

Agglutination tests using erythrocytes sensitized to penicillin at pH 9.0 showed—compared with the results found with pH 6.1 sensitized erythrocytes—a positive agglutination reaction in yet another serum from a penicillin allergic (BSJ)

TABLE 4

Frequency of Agglutinating Antibody to pH 6.1 Penicillin-Sensitized Erythrocytes in Penicillin Allergies and in Controls without Penicillin Allergy in Relation to Time of Last Exposure to Penicillin.

	Exposure to penicillin within past 3 mths.	Exposure to penicillin more than 3 mths. ago	No known exposure to penicillin
53 penicillin allergies	15/32	10/21	
12 pts. with alleged but not confirmed penicillin allergy	4/7	1/5	
120 pts. without a history of penicillin allergy	4/27	4/79	0/14

Number of sera with positive reaction / number of sera studied.

Blank: No patients studied.

and in 12 sera from patients without a history of penicillin allergy (Table 3). Thus, agglutination tests using pH 9.0 sensitized erythrocytes were less specific in predicting clinical penicillin allergy.

The sensitivity of the agglutination test was apparently increased by alkaline sensitization of the erythrocytes as regards sera with low titre values. At the same time, there was an almost constant reduction of titre values for heavily agglutinating sera. The technique of the two agglutination tests, described under items (1) and (2), however, did not differ only in respect to the pH during the sensitization of the erythrocytes. Sensitization times, sensitization of whole blood or of washed erythrocytes, and the use of normal serum or phosphate-buffered saline as diluent also differ in the two methods, and all these factors may have influenced the results.

The provocation tests confirmed that agglutinating antibody may occur, even in a high titre, in persons without signs of clinical penicillin allergy. Patients with a history of penicillin allergy and with positive agglutination reaction would occasionally tolerate penicillin therapy and patients without a history of penicillin allergy, but a positive agglutination reaction would usually tolerate penicillin therapy without exhibiting allergic reaction.

When the patients re-appeared for provocation tests, it was found, as might be expected, that the previous information about penicillin allergy had been to a marked extent unreliable, even though the patients had been thoroughly questioned.

Coombs' antiglobulin technique has been tried on several previous occasions in agglutination tests using penicillin-sensitized erythrocytes. Positive reactions in sera without directly demonstrable agglutinating antibody were originally reported by Ley et al. (50) in randomly selected sera. Subsequent studies, comprising also sera from penicillin allergics, have given only a very few positive (34) or else entirely negative results (35, 93). The results in the present study were also negative for sera from penicillin allergics without directly demonstrable agglutinating antibody. Therefore, the Coombs technique,

INHIBITION OF HAEMAGGLUTINATION WITH PENICILLIN-SENSITIZED ERYTHROCYTES
BY PRECEDING INCUBATION OF TEST SERUM WITH PENICILLIN.

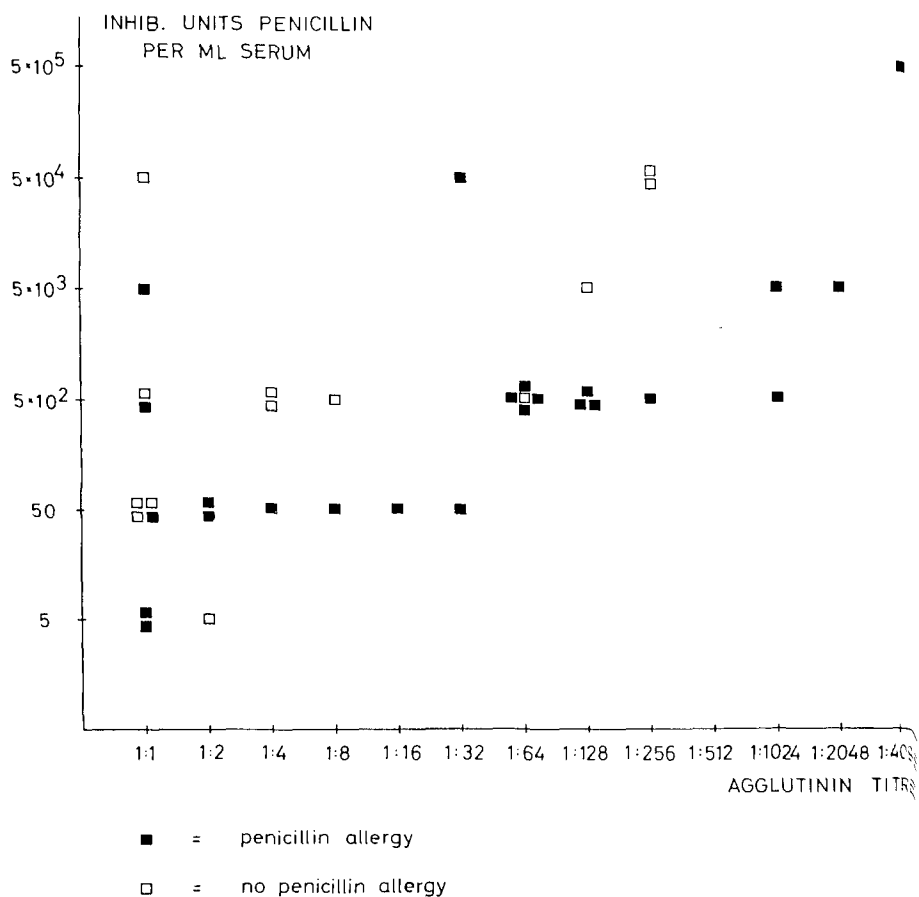


Fig. 2.

INHIBITION OF HAEMAGGLUTINATION WITH PENICILLIN-SENSITIZED ERYTHROCYTES
BY PRECEDING INCUBATION OF TEST SERUM WITH PENICILLIN.

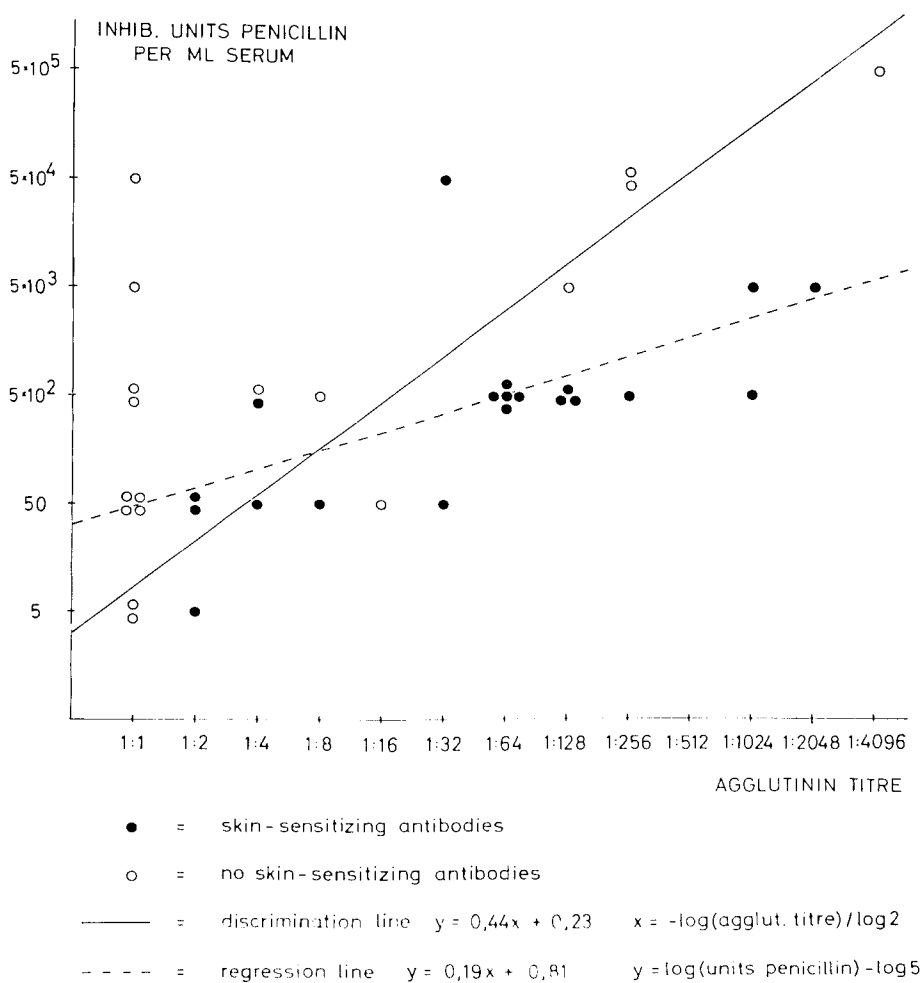


Fig. 3.

as used here, is not suited for improving the agglutination test with penicillin-sensitized erythrocytes.

Inhibition of the agglutination reaction using penicillin-sensitized erythrocytes by preceding incubation of the serum with penicillin has been described previously (5, 22, 50, 93). If inhibition is obtained, the agglutination reaction is proved to be specific. For some sera, a proportionality between the titre of agglutinating antibody and of the penicillin concentration required for inhibition has been reported (5), but there have also been reports on a few sera unexpectedly difficult to inhibit, in particular from patients who had not shown manifest penicillin allergy (7, 16, 69).

The present inhibition experiments (Fig. 2) showed for the 37 studied sera as a group no direct relationship between the agglutinin titre and the concentration of penicillin required for inhibition. However, sera from penicillin allergies showed a tendency to inhibition at a relatively lower concentration of penicillin than sera from patients without penicillin allergy (Fig. 2).

When considering sera with agglutinating as well as skin-sensitizing antibodies (Fig. 3), there is a significant correlation between the agglutinin titre and the concentration of penicillin needed for inhibition ($r = 0.66$ $p < 0.01$). The regression line is represented by $y = 0.19x + 0.81$ for $x = -\log$ (agglutinin titre)/ $\log 2$ and $y = \log$ (units penicillin) $- \log 5$. When applying a discriminant analysis to data for sera with skin-sensitizing antibodies against sera without skin-sensitizing antibodies, the line represented by $y = 0.44x + 0.23$ proves to afford the best statistical differentiation between the two groups.

Below the discrimination line $y = 0.44x + 0.23$ (Fig. 3) shows the parameters for:

- 14 penicillin allergies with skin-sensitizing antibodies.
- 4 penicillin allergies (MH, ASJ, JJN, and OMJ) without demonstrable skin-sensitizing antibodies.
- 1 patient with alleged but not confirmed penicillin allergy and no skin-sensitizing antibodies.

2 patients without a history of penicillin allergy, tolerating penicillin, but with a positive cutaneous reaction to penicilloyl-polylysine and penicilloyl-specific skin-sensitizing antibodies.

Above the discrimination line $y = 0.44x + 0.23$ Fig. 3 shows the parameters for:

5 patients without any evidence of past or present penicillin allergy.

4 patients with alleged but not confirmed penicillin allergy and no skin-sensitizing antibodies.

3 penicillin allergies without demonstrable skin-sensitizing antibodies (OMN, KED, and OBO).

1 patient without a history of penicillin allergy, tolerating penicillin, but with a positive cutaneous reaction to penicilloyl-polylysine and penicilloyl-specific skin-sensitizing antibodies.

3 penicillin allergies with skin-sensitizing antibodies (EMW, ELP, EF).

Thus, inhibition of haemagglutination, incubating serum with penicillin, makes it possible, to some extent, to distinguish between sera with and without simultaneous presence of skin-sensitizing antibodies. Above the line obtained by a discriminant analysis 4 out of 16 sera contained skin-sensitizing antibodies as compared with 16 out of 21 below the line.

Inhibition of agglutination reaction to penicillin-sensitized erythrocytes by preceding incubation of the serum with 2-mercaptoethanol has been described previously (29, 46, 82, 91). If the agglutination reaction is inhibited, the agglutinating antibody is supposed to be of the *Ig M* class, and if no inhibition occurs it is supposed to be *Ig G* antibody. The significance of the results has previously been secured by simultaneous investigations using ultracentrifugation or precipitation of agglutinating sera with specific anti-*Ig M* and anti-*Ig G* antibody (46).

The results of inhibition experiments with 2-mercaptoethanol in the present study appeared to be reliable for most sera. The findings indicated the presence of *Ig M* antibody in the

sera from KÅH, OEP, OMJ, and IV, of *Ig G* antibody in the sera from ASJ, EMT, and EMW and of *Ig M* + *Ig G* antibodies in the sera from EJN, HMP, EGC and DJ. For a few sera (MOP, KU, EJ 2, and EC) the results of inhibition experiments using 2-mercaptoethanol were highly variable from test to test without demonstrable cause. In particular, no errors or defects in the technique could be demonstrated. Accordingly, it was not possible to obtain a definite dissociation between agglutinating antibodies of the *Ig M* and *Ig G* classes by the technique employed.

However, the results suggest that the separation between sera with and sera without skin-sensitizing antibodies in Fig. 3 may be due to different classes of agglutinating antibodies. Thus, sera with sole *Ig G* antibodies are situated close to or above the discrimination line, whereas sera with *Ig M* antibodies are situated below.

Investigation for skin-sensitizing antibodies in the sera of 12 patients with alleged but not confirmed penicillin allergy gave negative results. Investigation of serum from 120 patients without a history of penicillin allergy gave positive results in 3, viz. penicilloyl-specific skin-sensitizing antibodies of a low titre. These patients also gave a positive cutaneous reaction to penicilloyl-polylysine but tolerated penicillin in a therapeutic dose.

As stated in the introduction, the demonstration of skin-sensitizing antibodies must generally be considered tantamount to clinically manifest allergy, and skin-sensitizing antibodies are not expected to be found in non-allergic persons. These hypotheses are largely confirmed by the present studies. Tolerance to penicillin in a therapeutic dose in 3 patients with penicilloyl-specific skin-sensitizing antibodies may be due to insufficient exposure to penicilloyl in the provocation with penicillin. As indicated by passive transfer tests (66) these 3 patients would presumably have developed a clinical allergic reaction to a higher dose of penicillin or to longer-lasting penicillin therapy.

For 53 patients with clinically manifest penicillin allergy,

TABLE 5
History, Clinical, and Serological Findings in 53 Patients with Definite Penicillin Allergy.

Patient	Family history of atopic disease	Personal history of atopic disease	No. of previous systemic treatm. w. penicillin	Occupational exposure	Months since last allergic reaction	Intra-cut. react. units penicillin immediate-type	Cutaneous react. penicillin delayed-type	Intra-cut. react. Mol. PPL immediate-type	Provocation test, reaction to units penicillin	Interval until sympt. on provoc.	Skin-sensitizing antibodies			Agglut. pH 6.1 penicillin sensit. erythroc.	Agglut. pH 9.0 penicillin sensit. erythroc.	Inhibition of agglut. units penicillin per ml serum
EMW	—	+	3	—	4	10^{-5}	+	$+10^{-18}$ M	Anaphyl. react. 10^{-5}	2'	1:1000	1:10	1:100	1:32	1:16	5×10^4
ELP	—	+	1	+	1	10^{-1}	—		Anaphyl. react. 10^{-1}	5'	1:10	—	—	1:2	1:2	50
OMN	+	+	10	—	1	—	—		Anaphyl. react. 5×10^{-1}	2'	—	—	—	1:1	1:4	5×10^2
EKS	—	—	20	—	1	10^{-3}	—	—	Anaphyl. react. 10^{-2}	2 h	1:100	—	1:10	—	—	
EJ ₁	++++	—	4	—	60	10^{-2}	—		Anaphyl. react. 10^2	2½ h	1:10	—	—	—	—	
MHJ	—	+	15	+	4	—	—	$+10^{-11}$ M§	Anaphyl. react. 10^{-1}	10'	1:1	1:1	—	1:32	—	50
EMT	—	—	2	—	12	1	—	$+10^{-7}$ M	Exanthema 1	1 h	—	1:10	—	1:2048	1:1024	5×10^2
ELC	+	+	1	+	1	—	—		Anaphyl. react. 1	3'	—	—	—	—	—	
HHM	++	—	2	—	1	—	—		Asthma 10	10'	—	—	—	—	—	
KU	+	—	9	+	1	10	—	$+10^{-12}$ M	Anaphyl. react. 10	40'	—	1:1000	1:10	1:64	1:16	5×10^2
EJ ₂	++	+	2	+	½	10	—	—	History of anaphyl.		—	1:100	—	1:64	1:16	5×10^2
AEL	—	+	40	—	48	—	—		Anaphyl. react. 10	5'	—	—	—	—	—	
KKT	—	+	15	—	24	—	—		Anaphyl. react. 10^2	2'	—	—	—	—	—	
JVN	—	—	1	—	¾	10^2	+		Erythema 10^2	72 h	1:10	—	—	—	—	
MAS*	—	—	0	—	¾	10^2	—		Urticaria 10^2	48 h	1:10	—	1:100	—	—	
OEP	—	+	0	—	1	10	—		Urticaria 10^2	2 h	1:10	1:100	1:1	1:128	1:16	5×10^2
OCC*	—	—	1	—	4	1	+		Urticaria 10^2	6 h	1:1	1:1	1:10	—	—	
EVP	—	—	0	+	0	—	+		Eczema 10^2	5 h	—	1:1	—	—	—	
EGC	—	—	8	—	1	10^2	—		History of anaphyl.		—	1:1	1:10	1:128	1:16	5×10^2
DJ	—	—	7	—	36	10^{-1}	—	$+10^{-10}$ M	Erythema 3×10^2	20'	1:10	1:100	1:10	1:64	1:32	5×10^2
EJN	—	—	2	—	¼	10^3	—		Urticaria 10^3	3 h	—	1:1000	1:100	1:1024	1:64	5×10^2
EMB	—	—	0	+	0	—	+		Eczema 10^3	4 h	—	—	—	—	—	
AMJ	+	—	3	+	1	—	+		Angioneurotic oedema 10^3	3 h	—	—	—	—	—	
ATJ*	+	+	10	—	24	—	—		Urticaria 10^3	3 h	—	—	—	—	—	
MH	—	—	0	—	48	—	—		Exanthema 10^4	5'	—	—	—	1:1	1:1	5
AK	+	+	8	—	2	—	—		Anaphyl. react. 10^3	3'	—	—	—	—	—	
ANT*	+	+	3	—	½	—	—		Eryth. multif. 10^3	2 h	—	—	—	—	—	
MOP	+	+	5	—	1	—	—	—	Asthma 10^3	2 h	1:1	—	—	1:128	1:32	5×10^2
HEN	—	—	2	—	36	10^3	—		History of exanthema		—	1:1	—	—	—	
IV	—	—	5	—	48	—	+		Anaphyl. react. 10^4	1'	1:10	1:1	—	1:8	1:2	50
KÄH	++	+	6	—	⅓	10^4	—		Asthma 10^4	5'	—	1:10	1:1	1:1024	1:32	5×10^3
AGJ	—	—	0	—	36	—	+		Urticaria 10^4	10 h	—	—	—	—	—	
FRB	+	+	0	+	0	—	+	—	Asthma 10^4	5'	—	—	—	—	—	
TP	—	—	1	—	12	—	—	—	Anaphyl. react. 10^4	2'	—	—	—	—	—	
JPP	—	—	2	—	1½	—	—		Anaphyl. react. 10^4	10'	—	—	—	—	—	
JMW	+	—	2	—	2	—	—		Rhinitis 10^4	3 h	—	—	—	—	—	
HP	—	—	12	—	1	—	—	$+10^{-9}$ M	Exanthema 10^4	14 h	—	1:10	—	—	—	
JEM	—	—	0	—	48	—	—	—	Eczema 10^4	24 h	—	—	—	—	—	
EC	++	—	4	+	0	—	+		Angioneurotic oedema 10^4	24 h	—	1:1	—	1:64	1:32	5×10^2
JS*	—	—	2	—	4	10^3	—	$+10^{-9}$ M	Urticaria 2×10^4	48 h	1:1	—	—	—	—	
MR	—	—	11	—	18	—	—		Urticaria 10^5	12 h	—	—	—	—	—	
KED	—	—	1	+	3	—	—	—	Asthma 10^5	3 h	—	—	—	1:1	1:2	50
MPA	—	+	4	—	72	10^4	—		Exanthema 10^5	3 h	—	—	—	1:2	1:2	
ASJ	—	—	0	—	1	—	+		Erythema 3×10^5	2 h	—	—	—	1:4096	1:2048	5×10^6
KLH	—	—	3	—	1	10^2	—		Urticaria 10^6	30'	1:1	—	—	—	—	
ALJ	++++	+	2	—	72	—	+	$+10^{-7}$ M	Urticaria 10^6	10'	—	1:100	—	1:4	1:4	50
HMP	—	+	3	—	1	—	+		Exanthema 10^6	4 h	—	1:10	1:1	1:256	1:256	5×10^2
EF	—	—	0	—	1	—	+		Urticaria 10^6	24 h	—	1:1	—	1:2	1:8	50
OBO	+	—	0	—	5	—	+	—	Exanthema 10^6	3 h	—	—	—	1:1	1:2	5×10^3
JJN	—	+	4	+	0	—	—	—	Rhinitis 10^6	24 h	—	—	—	1:1	1:1	5
OMJ	—	+	1	+	6	—	+	—	Angioneurotic oedema 10^6	10'	—	—	—	1:16	1:2	50
BSJ	—	—	3	—	½	—	—		No complaints		1:1	—	—	—	1:8	
EJ ₃	—	—	3	—	½	—	—		Pruritus 10^4	4 h	—	1:1	1:1	—	—	

++ = number of familial cases.
* = history of serum sickness-like reaction.
Blank = investigation not performed

' = minutes between provocation and allergic symptoms.
h = hours between provocation and allergic symptoms.
— = negative reaction.

PPL = penicilloyl-polylysine.
§ = after hyposensitization with penicillin.
+ = positive reaction.

the present results of agglutination experiments using penicillin-sensitized erythrocytes were compared with a previous investigation for skin-sensitizing antibodies (64) and with clinical parameters (68) for the same patients (Table 5). At first sight, the results of all immunological investigations, cutaneous tests, and serological studies present a motley picture. This suggests that allergic reactions to penicillin are series of clinical symptoms and signs elicited by different immune reactions to several chemical determinants.

Owing to the limited number of patients, showing many clinical types of reaction, difficulty must be expected in obtaining a significant correlation between the various parameters. *Without statistically significant correlation* higher titre values of penicillin-specific skin-sensitizing antibodies were observed in very sensitive penicillin allergics. There was a preponderance of a family and personal history of atopic diseases among patients with anaphylactic reactions and asthma—unlike the findings in allergic patients with urticaria, erythema, and exanthema—and a preponderance of positive delayed-type intracutaneous reaction to penicillin in patients with angioneurotic oedema.

With a statistical significance (χ^2 test) the following parameters were correlated.

Positive immediate-type cutaneous reaction to penicillin and the presence of skin-sensitizing antibodies on the whole ($P < 0.001$).

Positive immediate-type cutaneous reaction to penicillin and the presence of skin-sensitizing antibodies with a specificity for penicillin ($P < 0.01$) and penicilloate ($P < 0.001$).

Positive immediate-type cutaneous reaction to penicilloyl-polylysine and penicilloyl-specific skin-sensitizing antibodies ($P < 0.001$).

Skin-sensitizing antibodies to penicilloyl-polylysine and to benzylpenicilloate ($P < 0.001$).

Agglutinating antibodies and penicilloyl-specific skin-sensitizing antibodies ($P < 0.01$).

Agglutinating antibodies with titre value $\geq 1:32$ and penicilloyl-specific skin-sensitizing antibodies of a titre $\geq 1:10$ ($P < 0.01$).

On comparison with a previous study by *Redmond & Levine* (72) there was in a striking number of cases a discrepancy between the results of the immediate-type cutaneous tests and the investigations for skin-sensitizing antibodies. In some cases (EMT, KU, EJ 2, EGC, EJN, HEN, KÅH) this was presumably due to the eliciting of penicilloyl-specific reactions upon intracutaneous testing with benzylpenicillin as previously described in passive transfer tests (65).

Serologically, studies for skin-sensitizing and agglutinating antibodies afforded only an assumption of penicillin allergy in 37 out of 53 patients with definite penicillin allergy. Among 16 patients with entirely negative serological reactions 6 had anaphylactic reactions. Serological tests as performed in the present study cannot predict clinically manifest penicillin allergy with sufficient accuracy.

SUMMARY

Agglutinating antibodies to pH 6.1 penicillin-sensitized erythrocytes were demonstrated in 25 out of 53 sera from penicillin allergics, in 5 out of 12 sera from patients with alleged not confirmed penicillin allergy and in 8 out of 120 sera from patients without a history of penicillin allergy. Titre values ranged from 1:1 to 1:4096. The occurrence of agglutinating antibody was significantly more common in penicillin allergy, but not tantamount to such allergy.

Sensitization of erythrocytes by penicillin at pH 9.0 increased the sensitivity of the agglutination reaction to sera with low titre values but reduced the specificity of the method for predicting clinical penicillin allergy because of the many reactions among patients without a history of penicillin allergy.

It was not possible to increase the sensitivity of the agglutination reaction by using Coombs' anti-human globulin sera.

Inhibition of the agglutination reaction to penicillin-sensitized erythrocytes was possible in all cases by preceding incubation of the test serum with benzylpenicillin. For sera with agglutinating as well as skin-sensitizing antibodies there was a correlation between the agglutinin titre and the penicillin concentration needed for total inhibition of the agglutination.

In the inhibition experiments using penicillin it proved possible, to some extent, to distinguish between sera from patients with and without manifest penicillin allergy and between agglutinating sera with and without simultaneous presence of skin-sensitizing antibodies.

Inhibition of the agglutination reaction to penicillin-sensitized erythrocytes proved possible in some cases by preceding incubation of sera with 2-mercaptoethanol, but the results were not reproducible for all the sera. However, the investigation suggested that the separation of sera by inhibition experiments with penicillin could be explained from the occurrence of mainly Ig G antibody in sera from non-penicillin allergics without skin-sensitizing antibodies, and of Ig M antibody in sera from penicillin allergics with skin-sensitizing antibodies.

The results of the agglutination tests were compared with previously published determination of skin-sensitizing antibodies and clinical investigations on the same groups of patients. This showed a significant correlation between the occurrence of agglutinating antibody and penicilloyl-specific skin-sensitizing antibody as well as between a positive immediate-type intracutaneous reaction to penicillin and demonstrable skin-sensitizing antibodies.

Serological investigations with determination of agglutinating and skin-sensitizing antibodies rendered it possible to make an assumed diagnosis of penicillin allergy in only 37 out of 53 patients with manifest penicillin allergy.

ACKNOWLEDGEMENTS

The studies were aided by grants from Statens Almindelige Videnskabsfond, Thorvald Madsens legat and frk. P. A. Brandts legat.

The author is indebted to *Herdis von Magnus*, M.D., Head of the Enterovirus

Department, and to *Jørgen Andersen*, M.D., Head of the Blood Bank and Blood Grouping Department, Statens Serum Institut, Copenhagen, for help in the experiments with passive transfer to monkey skin and with Coombs' antihuman-globulin technique.

Thanks are due to cand. act. *Jørgen Nyboe* for assistance in the statistical calculation.

In the agglutination experiments the author enjoyed the careful technical assistance of Miss. *Susan Wells*, Miss. *Hanne Clausen* and Miss. *Merete Sørensen*.

REFERENCES

1. *Ackroyd, J. F.*: Clin. Sci. 13, 409, 1954.
2. *Arbesman, C. E., Kantor, S. Z., Rose, N. R. & Witebsky, E.*: J. Allergy 31, 257, 1960.
3. *Arbesman, C. E., Rose, N. R., Kantor, S. Z. & Beede, R. B.*: J. Allergy 31, 317, 1960.
4. *Arbesman, C. E., Girard, P. & Rose, N. R.*: J. Allergy 35, 535, 1964.
5. *van Arsdel, P. P., O'Rourke, T. K., Horan, J. D. & Kumasaka, Y.*: J.A.M.A. 185, 584, 1963.
6. *van Arsdel, P. P., Tohe, A. D. & Pasnick, L. J.*: J. Allergy 34, 526, 1963.
7. *van Arsdel, P. P. & Gilliland, B. C.*: J. Lab. Clin. Med. 65, 277, 1965.
8. *Beardwell, C. G.*: Proc. Royal Soc. Med. 57, 332, 1964.
9. *Bendixen, G.*: Ann. Int. Med. 64, 668, 1966.
10. *de Besche, A.*: Norsk Mag. Lægevidensk. 92, 17, 1931.
11. *Bierman, C. W. & van Arsdel, P. P.*: J. Allergy 43, 267, 1969.
12. *Bobitt, J. R., Schechter, H. & Pollak, V. E.*: Proc. Soc. Exp. Biol. & Med. 117, 608, 1964.
13. *Budd, M. A., Parker, C. W. & Norden, C. W.*: J.A.M.A. 190, 203, 1964.
14. *Call, R. A. & Gilbert, R. A.*: J.A.M.A. 134, 1475, 1947.
15. *Callaway, J. L. & Barefoot, S. W.*: J. Invest. Derm. 7, 285, 1946.
16. *Clayton, E. M., Altshuler, J. & Boøe, J. R.*: Am. J. Clin. Path. 44, 648, 1965.
17. *Connell, J. T. & Sherman, W. B.*: J. Allergy 35, 18, 1964.
18. *Connell, J. T. & Sherman, W. B.*: J. Allergy 35, 169, 1964.
19. *Cooke, P. A., Menzel, A., Meyers, P., Skaggs, J. & Zeman, H.*: J. Allergy 27, 324, 1956.
20. *Criep, L. H.*: J.A.M.A. 126, 429, 1944.
21. *McElfresh, A. E. & Huang, N. N.*: New Engl. J. Med. 266, 246, 1962.
22. *Epp, M.*: Science 130, 1472, 1959.
23. *Fang, Yeh*: Chinese Med. J. 76, 599, 1958.
24. *Fellner, M. J. & Ripps, G. S.*: Clin. Res. 14, 267, 1966.
25. *Fitzgerald, J. D. L. & Irvine, A. H.*: Canad. M.A.J. 74, 46, 1956.
26. *Freedman, A. L., Barr, P. S. & Brody, E. A.*: Am. J. Med. 20, 806, 1956.
27. *Friedlaender, S. & Friedlaender, A. S.*: J. Allergy 35, 361, 1964.

28. *Frugoni, C.*: Il. Policlinico 29, 179, 1922.
29. *Fundenberg, H. H. & German, J. L.*: Blood 15, 683, 1960.
30. *Gill, F. A.*: J. Immunol. 98, 778, 1967.
31. *Goldman, L., Friend, F. & Mason, L. M.*: J.A.M.A. 131, 883, 1946.
32. *Gyenes, L., Gordon, J. & Schon, A. H.*: Immunology 4, 177, 1961.
33. *Halpern, B., Ky, N. T. & Amache, N.*: J. Allergy 40, 168, 1967.
34. *Harris, J. & Faughan, J. H.*: J. Allergy 32, 119, 1961.
35. *Heggie, A. D.*: New Engl. J. Med. 262, 1160, 1960.
36. *Heremans, J. F., Faerman, J. P. & Faerman, C.*: J. Immunol. 91, 11, 1963.
37. *Hopkins, J. G. & Lawrence, H.*: J. Allergy 18, 251, 1947.
38. *Katz, H. J., Gill, K. A., Baxter, D. L. & Moschella, S. L.*: J.A.M.A. 188, 351, 1964.
39. *Keefer, C. S., Blake, F. G., Marshall, E. K., Lockwood, J. S. & Wood, W. B.*: J.A.M.A. 122, 1217, 1943.
40. *Kobayashi, M., Stahmann, M. A., Rankin, J. & Dickie, H. A.*: Proc. Soc. Exp. Biol. & Med. 113, 472, 1963.
41. *Kuhns, W. J.*: J. Exp. Med. 97, 903, 1953.
42. *Kunz, M. L., Reisman, R. E. & Arbesman, C. E.*: J. Allergy 40, 135, 1967.
43. *Käss, E. & Odegaard, A. E.*: Tidsskrift for Norsk Lægeforen. 79, 195, 1959.
44. *Levine, B. B. & Fellner, M. J.*: J. Clin. Invest. 44, 1067, 1965.
45. *Levine, B. B., Fellner, M. J. & Lezytska, V.*: J. Immunol. 96, 707, 1966.
46. *Levine, B. B., Fellner, M. J., Lezytska, V., Franklin, E. C. & Alisberg, N.*: J. Immunol. 96, 719, 1966.
47. *Levine, B. B. & Redmond, A. P.*: Int. Arch. Allergy 35, 445, 1969.
48. *Levine, B. B. & Zolow, D. M.*: J. Allergy 43, 231, 1969.
49. *Ley, A. B., Cahan, A. & Mayer, K.*: VII Congr. of Blood Transf. p. 539, Rome 1958.
50. *Ley, A. B., Harris, J. P., Brinkley, M., Liles, B., Jack, J. A. & Cahan, A.*: Science 127, 1118, 1958.
51. *Linde, S.*: Sv. Läkartidn. 53, 465, 1956.
52. *Ljaljević, M.*: Acta Allergologica 14, 193, 1959.
53. *Lyons, C.*: J.A.M.A. 123, 1007, 1943.
54. *Löhe, H. & Teller, H.*: Dermat. Wschr. 119, 645, 1947.
55. *Maganzini, H. C.*: New Engl. J. Med. 256, 52, 1957.
56. *Matheson, A. & Elegant, L.*: J. Allergy 26, 415, 1955.
57. *Mayer, P. S., Mosko, M. M., Schutz, P. J., Osterman, F. A., Steen, L. H. & Baher, L. A.*: J.A.M.A. 151, 351, 1953.
58. *Mendes, E., Rodrigues, A., Fernandes, A., Cintra, A. U. & Lacaz, C. S.*: Acta Allergologica 14, 445, 1959.
59. *Moeschlin, S. & Wagner, K.*: Schw. Med. Wschr. 1079, 1952.
60. *Moore, J. E., Mahoney, J. F., Schwartz, W., Sternberg, T. & Wood, B.*: J.A.M.A. 126, 67, 1944.
61. *Mueiling, R. J., Beren, T., Samson, R. F., Jencerein, E. P. & Guillory, J. D.*: Am. J. Clin. Pathol. 29, 503, 1958.

62. Nesmith, L. W. & Davis, J. W.: J.A.M.A. 203, 27, 1968.
63. Pedersen-Bjergaard, J.: Acta Allergol. 22, 299, 1967.
64. Pedersen-Bjergaard, J.: Acta Allergol. 24, 57, 1969.
65. Pedersen-Bjergaard, J.: Acta Allergol. 24, 87, 1969.
66. Pedersen-Bjergaard, J.: Acta Allergol. 24, 303, 1969.
67. Pedersen-Bjergaard, J.: Acta Allergol. 24, 333, 1969.
68. Pedersen-Bjergaard, J.: Acta Allergol. 25, 89, 1970.
69. Petz, L. D. & Funderberg, H. H.: New Engl. J. Med. 274, 171, 1966.
70. Prausnitz, C. & Küstner, H.: Centralbl. f. Bakt. 86, 160, 1921.
71. Radermecker, M. & Salmon, J.: Acta Allergol. 21, 285, 1966.
72. Redmond, A. P. & Levine, B. B.: J. Allergy 39, 51, 1967.
73. Reed, C. E., Sosman, A. & Barbee, R. A.: J.A.M.A. 193, 261, 1965.
74. Reisman, R. E., Rose, N. R., Whitebsky, E. & Arbesman, C. E.: J. Allergy 33, 178, 1962.
75. Rich, A. R. & Lewis, M. R.: Bull. Johns Hopk. Hosp. 50, 115, 1932.
76. Rorsman, H.: Acta Dermato-Vener. 37, 121, 1957.
77. Rosenberg, D. H. & Arling, P. A.: J.A.M.A. 125, 1011, 1944.
78. Salvaggio, J. E., Buechner, H. A., Seabury, J. H. & Arquembourg, P.: Ann. Int. Med. 64, 748, 1966.
79. Saperstein, S., Anderson, D. W., Goldman, A. S. & Kniker, W. T.: Pediatrics 32, 580, 1963.
80. Sarkany, I.: Lancet I, 743, 1967.
81. Schuppli, R.: Schw. Med. Wschr. 81, 589, 1951.
82. Schwartz, R. H. & Vaughan, J. H.: J.A.M.A. 186, 1151, 1963.
83. Shelley, W. B. & Juhlin, L.: Nature 191, 1056, 1961.
84. Shelley, W. B.: J.A.M.A. 184, 171, 1963.
85. Shelley, W. B. & Comaish, J. S.: J.A.M.A. 192, 36, 1965.
86. Siegel, B. B.: J. Allergy 33, 349, 1962.
87. Siegel, B. B. & Levine, B. B.: J. Allergy 35, 488, 1964.
88. Spain, D. M. & Clark, T. B.: Ann. Int. Med. 25, 732, 1946.
89. Strumia, P. V. & Raymond, F. D.: Arch. Int. Med. 109, 603, 1962.
90. Soborg, M. & Bendixen, G.: Acta Med. Scand. 181, 247, 1967.
91. Thiel, J. A., Mitchell, S. & Parker, C. W.: J. Allergy 35, 399, 1964.
92. Truitt, G. W.: Ann. Allergy 4, 196, 1946.
93. Watson, K. C., Joubert, S. M. & Bennett, M. A. E.: Nature 183, 468, 1959.
94. Watson, K. C., Joubert, S. M. & Bennett, M. A. E.: Immunology 3, 1, 1960.
95. de Weck, A. L.: Nature 202, 975, 1964.
96. de Weck, A. L. & Blum, G.: Int. Arch. Allergy 27, 221, 1965.
97. Welch, H., Lewis, C. N., Weinstein, H. I. & Boeckman, B. B.: Antibiot. Med. 4, 800, 1957.
98. Wide, L., Bennich, H. & Johansson, S. G. O.: Lancet II, 1105, 1967.
99. Yagi, Y., Maier, P., Pressman, D., Arbesman, C. E. & Reisman, R. E.: J. Immunol. 91, 83, 1963.
100. Zabel, H., Vogt, A. & Zabel, R.: Deutsc. Gesundh. 20, 1457, 1965.