

IMMUNOLOGIC REACTIONS TO PENICILLIN

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HYPERSENSITIVITY to penicillin has been observed during recent years in increasing frequency. Penicillin skin tests have proved to be of value primarily in identifying some of the individuals liable to immediate anaphylaxis. Presence or absence of skin reactivity has not, however, been of prognostic value in respect to later development of serum sickness, delayed urticaria, or other skin eruptions. In 1958 Ley and collaborators¹ reported that penicillin-coated erythrocytes were agglutinated by the sera of some individuals who have been treated with penicillin. He did not relate the hemagglutinin to skin sensitizing antibody, nor did he particularly implicate it in sensitivity reactions. Nevertheless, it is our opinion that he might have provided a new tool useful for the investigation of penicillin hypersensitivity. Therefore, we have used the hemagglutination test to examine the sera of patients observed to have had penicillin reactions. Comparison was made with the sera of patients recently treated with penicillin, but suffering no reactions, and with the sera of random patients with various other diseases.

METHODS

Preparation of Cells.—Fifty milliliters of blood was collected from a type “O” donor and added to 12 ml. of ACD† solution. The red cells were “penicillinized” by adding 6 ml. of the citrated blood to 200,000 units of penicillin G powder. This was incubated 1 hour at 37° C. and stored in the refrigerator. Portions of the penicillinized cells were removed, washed three times with pH 7.3 phosphate buffered saline, and re-suspended as 2 per cent or 5 per cent cell suspensions. The 2 per cent cell suspension was used for screening tests. The 5 per cent suspension was used in titrations in which the penicillinized cells were subdivided into aliquots after exposure to antibody, preparatory to differential Coombs tests. Control suspensions of washed “O” cells which had not been exposed to penicillin were employed in all experiments.

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†Per 100 ml.: 1.47 Gm. dextrose; 1.32 Gm. sodium citrate; 440 mg. citric acid.

Hemagglutination.—As a screening procedure, 0.05 ml. of a 2 per cent cell suspension was added to 0.3 ml. of serum. The serum and cells were then incubated at room temperature for fifteen minutes with gentle agitation, centrifuged at 800 r. p. m. for one minute, and read directly for agglutination. The cells were then washed three times with normal saline and re-suspended in a modified “Coombs” serum having specificity either for human γ_2 -globulin or for serum globulins other than γ_2 -globulins.^{2, 3} These then were allowed to stand at room temperature fifteen minutes, centrifuged gently, and read again for hemagglutination. The use of the “Coombs” serum was designed to detect instances in which antibodies were present, but in which the antibodies for quantitative or qualitative reasons failed to provide direct agglutination. In other instances, the Coombs technique was used to enhance agglutination already seen to some degree.

TABLE I. POSITIVE HEMAGGLUTINATION REACTIONS

DIAGNOSIS	INCIDENCE
Group 1. “Probable” penicillin reactions	6/16
Group 2. “Suspected” penicillin reactions	0/5
Group 3. Rheumatic fever patients on oral penicillin	3/307
Group 4. Random ward subjects	0/167
Group 5. Patients recently completing a course of penicillin (off drug 2-7 days)	0/32
Group 6. Collagen-vascular disease	4/13
Total	13/541

Skin Testing.—One to two hundredths milliliter of a freshly prepared solution containing 1,000 units of penicillin G per milliliter of saline was injected intracutaneously. A control site was injected with an equal amount of saline. Both sites were read at the end of fifteen to twenty minutes. If no reaction was observed, 0.01 ml. of a solution containing 10,000 units penicillin G per milliliter was injected intracutaneously and the skin again observed for fifteen to twenty minutes. If the reactions were still negative, the sites were again read at the end of twenty-four hours. Patch tests were performed with an ointment containing 100,000 units penicillin G powder per gram of water-miscible base. Tested areas were observed at intervals of twenty-four and forty-eight hours.

RESULTS

Clinical Observations.—Of 541 serum samples examined, 13 gave positive hemagglutinin patterns: six of sixteen patients with a diagnosis of “probable” penicillin reaction, three of 307 rheumatic fever patients on oral penicillin prophylaxis, and four of thirteen patients with “collagen-vascular” disease. None of 167 random ward subjects, none of thirty-two selected ward patients recently completing courses of penicillin, and none of five patients “suspected” of having had a penicillin reaction had positive reactions. Separation of patients into “probable” and “suspected” categories of penicillin reactions was necessitated by the obvious difficulty of establishing definite clinical

diagnoses in patients who often were receiving more than one drug, and in whom the adverse clinical picture might also have represented an unusual expression of the underlying disease rather than a drug reaction.

Thirteen of the sixteen individuals experiencing probable penicillin reactions (Group 1) were also skin tested. A comparison of these skin test results with the penicillin hemagglutination test was made (Table II). Of four individuals with an erythematous macular rash, one gave an immediate skin reaction and another gave a delayed erythema in twenty-four hours.

TABLE II. "PROBABLE" PENICILLIN REACTIONS

CLINICAL PICTURE	NUMBER OF PATIENTS	POSITIVE HEMAGGLUTINATION	POSITIVE SKIN TEST			TOTAL POSITIVE
			IMMED.	24 HR.	PATCH	
Erythematous macular rash	6	1/6	1/4	1/4	0/1	3/6
Exfoliative dermatitis	3	2/3	1/2	1/2		3/3
Urticaria	4	1/4	3/4	1/4	0/4	4/4
Angioneurotic edema	1	0/1	0/1	1/1	0/1	1/1
Serum sickness*	2	2/2	1/2	0/2	0/2	2/2
Totals	16	6/16	6/13	4/13	0/8	13/16

*Fever, arthralgia, lymphadenopathy, and urticaria.

Neither of these two individuals had the one positive hemagglutinin reaction seen in this subgroup. One of the three individuals with exfoliative dermatitis had a positive immediate skin reaction and also gave a positive hemagglutinin reaction. Another with exfoliative dermatitis showed delayed erythema and edema in skin testing and was negative in the hemagglutinin test. Three of four individuals with urticaria gave positive immediate skin reactions; the fourth gave a delayed (twenty-four hour) erythema and edema. Only one of these four patients gave positive hemagglutination. The one patient with angioneurotic edema gave only a delayed erythema on skin test. One of two patients with serum sickness gave a wheal and flare when skin tested shortly after her initial episode. The other patient, although negative by skin test immediately after her reaction, was positive with a strong wheal and flare response two months later. Both patients with serum sickness had positive hemagglutination reactions.

TABLE III. HEMAGGLUTINATION VS SKIN TESTS

HEMAGGLUTINATION		SKIN TEST POSITIVE	
		IMMEDIATE	DELAYED
Positive	6	3	0
Negative	10	4	4

By the combined use of skin testing and serum hemagglutination, thirteen of the sixteen patients with probable penicillin reactions were shown to have developed an immunologic response to the drug. Of 8 patch tests performed, none gave a positive reading. The lack of identity of positive skin reactivity with presence of penicillin hemagglutinin is emphasized in Table III. Only

half the patients with demonstrable hemagglutinin also had positive skin tests, and positive skin tests were seen in the absence of hemagglutination.¹

Penicillin hemagglutination was occasionally found in the sera of individuals who had been treated with penicillin but who had not been thought to have had a sensitivity reaction. The strengths of these hemagglutination reactions were generally less than those seen in the sera of patients diagnosed as having experienced penicillin reactions. The three patients with rheumatic fever on prophylactic penicillin were in this category. On review of their records, there was nothing to suggest a hypersensitivity reaction, either by the occurrence of skin eruptions or by the development of joint complaints, fever, or other symptoms consistent either with serum sickness or recurrent rheumatic activity.

Originally only the first five groups of patients were designated in this study. Group 6, "the collagen-vascular diseases," was established by virtue of the findings in two of our cases. The first was a 21-year-old girl, who had had a past history of vague arthralgias and suggestive blanching of the hands on exposure to cold, and who developed a blotchy, erythematous, infarctive skin lesion after a course of 10,000,000 units of penicillin given because of the incidental finding of a positive serological test for syphilis. Tests for treponema immobilizing antibody, however, were negative on repeated occasions. A very strong penicillin hemagglutination titer was noted some two months after the penicillin therapy and diminished slowly in titer for over the subsequent year. Angiitic skin lesions, demonstrated by biopsy, persisted in undiminished form during the year. The L.E. cell test was negative, but the diagnosis of lupus erythematosus has been strongly considered. It was felt that the development of the immune reaction to penicillin probably contributed significantly to the adverse course of vasculitis in this patient, but that the penicillin reaction was not the primary cause of the vasculitis. This patient had originally been classified in Group 4 of Table I, the "penicillin reactors." She was removed to Group 6 after recognition of the second patient, a 69-year-old woman with a clinical picture of rheumatoid arthritis, bronchopneumonia, and a positive L.E. cell test. This second patient had no clear-cut evidence of a penicillin reaction but had a positive hemagglutination reaction. She had been originally classified in Group 3 ("selected patients recently treated with penicillin").

Because of the interest engendered by these two latter patients, eleven sera from patients with established diagnoses of lupus erythematosus were taken from deep freeze storage and examined for penicillin antibodies. Two of these gave strong reactions. Review of the records of these patients revealed no definite clinical indications of penicillin reactions in them, although the lupus erythematosus was active in both prior to, during, and after the penicillin therapy. The eleven sera from patients with lupus erythematosus, together with the two above, have been grouped and constitute the thirteen sera of Group 6 of Table I.

Laboratory Observations.—Of the 13 sera positive for penicillin antibody, 12 demonstrated direct agglutination. Six were positive by “Coombs” test; in one of these the antibody was detected only by the “Coombs” technique.

Differential studies were performed with absorbed “Coombs” sera. One of the “Coombs” sera (anti- γ_2 -globulin) had been prepared by injection of rabbits with γ_2 -globulin (Fraction II-1,2) and absorbed with an agammaglobulinemic human serum. This serum reacted strongly with an Rh-positive cell coated with anti-Rh antibody, but failed to react with a cell coated with complement-fixing incomplete cold agglutinin.^{2, 3} The other Coombs serum (anti-non- γ_2 -globulin) was from a rabbit that had been injected with whole human serum and had been optimally absorbed with Fraction II-1,2. It failed to agglutinate Rh positive cells sensitized with anti-Rh antibody, but agglutinated strongly cells coated with incomplete cold agglutinin. Anti-non- γ_2 -globulin sera have been shown to detect complement protein.^{2, 3} These absorbed “Coombs” sera were used to test penicillinized cells that had been exposed to the patients’ antibody and then rewashed three times in saline. Generally, any original strong agglutination of the penicillinized cells in a patient’s serum was considerably reduced or obliterated by the washing procedure. Redevelopment of significantly strong agglutination was noted in the six sera indicated in Table IV. In three instances, agglutination reactions were noted in the anti- γ_2 -globulin serum, consistent with the assumption that the penicillin hemagglutinin in these instances was a gamma globulin. In three other instances, agglutination developed in the anti-non- γ_2 -globulin. Consistent with the assumption that these latter reactions depended upon complement fixation on the sensitized cell was the observation in one of the three (Johnson; Table IV) that hemagglutination activity was lost with aging and that the activity could be recovered by the addition to the aged serum of fresh normal serum containing no hemagglutinating antibody.

TABLE IV

DIRECT AGGLUTINATION	AGGLUTINATION ON RESUSPENDING THE WASHED CELLS IN:					
	SALINE	ANTI- γ_2 -GLOBULIN		ANTI-NON- γ_2 -GLOBULIN		
		1/50	1/200	1/20	1/100	
Knittle	+++	++	+++	+++	+	+
DiBartolo	++	+	++	++	—	—
Levin	++	±	+++	+++	—	—
Johnson	++	—	—	—	++	++
Siddons	+++	±	—	—	+++	+++
Rogoff	—	—	—	—	++	++

The specificity of the penicillin hemagglutination reaction was well established by Ley in his original publication,¹ both by inhibition with penicillin and by the effect of penicillinase on the system. These same criteria for specificity were employed also in the present study. Even small amounts of penicillin added to test sera prior to addition of the penicillinized cells were capable of completely inhibiting the reaction of the sera with the cells (Table V), and penicillinase abolished the ability of penicillin to coat red cells for hemagglutination with these sera.

The synthetic penicillin, Syncillin (Penicillin-152), also could inhibit serum hemagglutination in some instances (Table V) quite as effectively as penicillin G. With some other sera, however, Syncillin was inactive in inhibition. In all inhibition studies, the cells were coated with penicillin G, and inhibition was assessed by preincubating the antibody-containing serum with the penicillin preparation. Failure of Syncillin to inhibit agglutination by a given serum thus would suggest that the antibody activity in that serum was predominantly for the side group (R-group, Fig. 1) rather than the basic penicillin radicle.

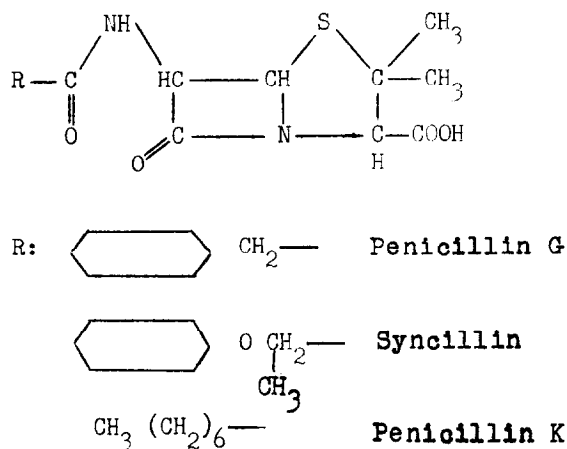


Fig. 1.—Penicillin.

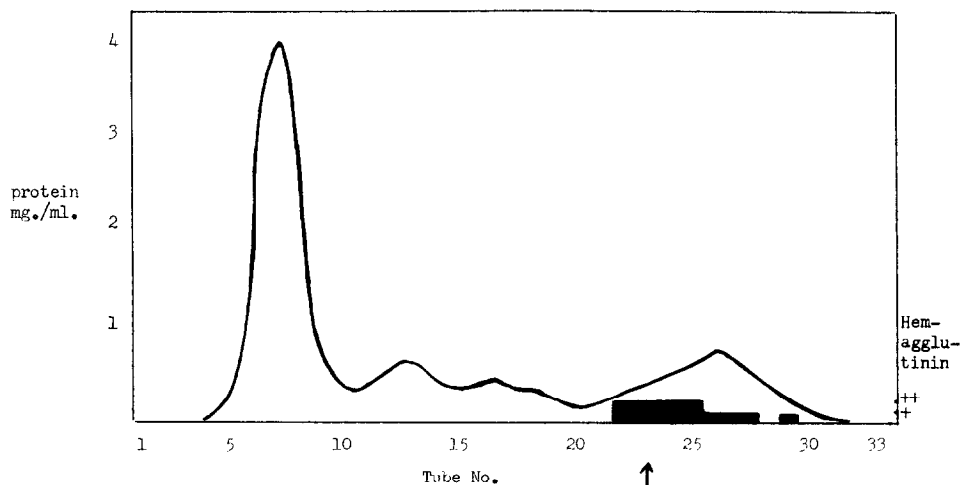


Fig. 2.—Penicillin hemagglutinin. Starch block electrophoresis of serum (Knittle). Serum was applied at the arrow (↑) and the anode was at the left. Barbitol buffer was used at pH 8.6, 0.075 M. Half-inch blocks were cut out, eluted with 0.15 M NaCl into separate tubes, and tested individually for protein content (—) and hemagglutinating activity (■).

Zone electrophoresis in potato starch at pH 8.6 has revealed the hemagglutinin to migrate as a fast γ -globulin on two occasions. One of these was with the serum Knittle (Table IV and Fig. 2). The other was with a serum which hemagglutinated but failed to give a Coombs reaction.

TABLE V. INHIBITION OF AGGLUTINATION OF PENICILLIN-COATED CELLS

PER TUBE	ANTI-PENICILLIN SERUM (WALKER*)				
	UNDIL.	1:2	1:4	1:8	1:16
Penicillin					
100 u.	-	-	-	-	-
50 u.	±	-	-	-	-
20 u.	+	+	+	±	-
2 u.	++	++	++	+	-
0 u.	++	++	++	+	-
Syncillin					
125 u.		-	-	-	-
60 u.		+	-	-	-
30 u.		+	-	-	-
15 u.		+	±	-	-
0 u.		++	++	+	-

*A 74-year-old man who developed a desquamating rash from procaine penicillin G given for a pneumonitis developing during therapy for congestive heart failure. Recovery was uneventful.

DISCUSSION

The present small experience in studying penicillin reactions has emphasized how limited is the skin test response alone as a diagnostic tool in the identification of allergy. Lack of positive skin reactions in patients clinically hypersensitive to pollens has led more to attempts at variations in skin testing than to trying basically different methods of assessment of serum antibody or of cellular hypersensitivity. The infrequency of positive skin reactions in drug hypersensitivity has prompted some to the belief that most drug reactions have no immunologic basis, and impetus to this has been provided by the finding of a genetically-determined enzyme defect in primaquine sensitivity. In the current state of activity in the employment of hemagglutination reactions for the assessment of "desensitization," it may be worth some thought that these may have even greater usefulness in diagnosis. Gordon's⁴ finding (also Arbesman⁵) of positive ragweed hemagglutination reactions in patients who have had no prior treatment supports this possibility.

In most of the individuals reported in the present study who had experienced penicillin hypersensitivity reactions, it was possible to implicate a hypersensitive state either by positive skin tests or by positive hemagglutination reactions. Of thirteen individuals with a diagnosis of probable penicillin reaction who were tested by *both* criteria, only one was negative by both. Nevertheless, in more extensive testing, more cases certainly will continue to come to light, in which the currently employed criteria for hypersensitivity will demonstrate nothing, in spite of the clinical likelihood that a penicillin reaction has occurred. The development of still newer techniques for identifying the hypersensitive state should be sought for such situations. Just as the penicillin hemagglutination reaction has enhanced our present ability to confirm penicillin allergy in individuals with negative skin tests, so still newer testing methods may in the future be expected to improve further our diagnostic abilities.

As far as the character of penicillin antibody itself is concerned, our experience would indicate that it has the same diversity as has been found for

antibody to blood group antigens. Individual patients vary from one another in the type of immune response they make. Some have elaborated a non-complement-fixing antibody. Others have elaborated antibody that does fix complement. Sometimes direct hemagglutination occurred and the subsequent "Coombs" test was negative. Fudenberg⁶ has recently noted that penicillin hemagglutinin is found to be a 19S γ -globulin in some patients and a 7S in others. Neither Fudenberg nor ourselves, however, have observed hemagglutination attributable to a "pre-albumin," as described by Watson.⁷ Some patients have developed no detectable serum antibody, but have shown delayed skin reactivity. The specificity of such delayed reactions has not yet been established by the use of penicillinase or synthetic penicillin, but such specificity has been reported for the immediate wheal and flare response. The numbers of patients are as yet too small to indicate a predominate clinical picture corresponding to one or another of these types of immune response.

There is presently no particular reason to emphasize one type of penicillin antibody over another in respect to pathogenetic potential. Indeed current literature emphasizes the occurrence of the hemagglutination reaction with the sera of individuals suffering no apparent hypersensitivity reaction to the drug. Also patients with positive skin tests have been found who subsequently have tolerated penicillin therapy without mishap.⁹ Nevertheless, the incidence of positive hemagglutination reactions and of positive skin tests is definitely higher among those who have had penicillin reactions. The higher incidence of positive hemagglutinations among those having reactions has also been found by Heggie.⁸ There are quantitative as well as qualitative immunologic factors that are important in determining whether a given antibody response or other change in the immunologic state may permit hypersensitivity symptoms. In addition, there are undoubtedly non-immunologic determinants, presently poorly understood or not appreciated at all, which need description. Possibly some consideration should be given in this respect to the biochemical pathways involved in the substrate tissues and to corresponding alterations in the "intermediates" in the antigen-antibody interaction. The role of previously established disease in modifying the substrate response also needs attention, and more experience among patients with the collagen-vascular group of diseases would be of particular interest.

The specificity of the hemagglutinating antibody for penicillin is confirmed by (a) the ability of small amounts of penicillin G to inhibit the serum reaction, (b) the inability of penicillinase-treated penicillin to coat cells effectively for hemagglutination with known positive sera, and (c) the ability of synthetic penicillin (Penicillin-152) also to inhibit the reaction. This last observation is of additional significance in its implications concerning the use of synthetic penicillin as a substitute antibiotic in patients clinically sensitive to ordinary penicillin preparations. Although some cases may be found in which the synthetic preparation can be used without difficulty, there is no assurance of the safety of this; and the risk must be considered significant.

SUMMARY

Immunologic evidence of penicillin hypersensitivity can frequently be found in patients experiencing penicillin reactions. There is no good correlation between positive skin tests and positive hemagglutinations, each occurring independently of the other. There exist instances of penicillin hypersensitivity based on immune mechanisms other than skin sensitizing antibody, a fact that emphasizes the limitations of the use of skin tests alone for diagnosing allergic conditions. The hemagglutination technique has a high degree of specificity for the penicillin molecule.

ADDENDUM

Recent reports of Levine, B. B. (*J. Exper. Med.* 112: 1131, 1960) and of de Week, A. L., and Eisen, H. N. (*J. Exper. Med.* 112: 1227, 1960) indicate that a penicillin metabolite, rather than penicillin itself, may be the active constituent.

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