

EXPERIMENTAL SENSITIZATION WITH PENICILLINASE

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A RECENT study by Becker¹ described the action of penicillinase on circulating penicillin *in vivo* in guinea pigs and in human beings. It was found that parenteral administration of penicillinase inactivated all circulating penicillin, both in guinea pigs and in human beings. The therapeutic use of purified penicillinase for inactivation of circulating penicillin in patients suffering allergic reactions to penicillin was suggested.

After repeated injections of penicillinase in guinea pigs neither toxic, hypersensitive, nor anaphylactic responses were observed.¹ However, sensitization of rabbits with penicillinase was reported earlier by Perlstein and Liebmann,² as well as by Housewright and Henry.³ These authors found considerable amounts of specific precipitins in the sera of immunized rabbits.

The purpose of the present study was to re-examine the antigenicity of penicillinase in guinea pigs and rabbits and, if sensitization was produced, to determine the immunologic properties of their sera.

EXPERIMENTAL TECHNIQUES

Antigen.—Injectable penicillinase* is obtained from cultures of *Bacillus cereus*, strain NRRL-B 569. It is a purified, nonpyrogenic grade of enzyme in lyophilized form. Its potency is expressed in Schenley units: 1 unit of penicillinase will inactivate 1 unit of penicillin within one minute and 60 units of penicillin within one hour at 25° C. at a pH of 7.4. Our determinations have shown that 1,000,000 units of penicillinase contain 3 to 5 mg. of phosphotungstic-precipitable nitrogen.

Sensitization.—Male albino guinea pigs, each weighing 350 to 400 grams, and male albino New Zealand rabbits, each weighing 2,500 grams, were used for sensitization.

Group I: Nine guinea pigs and two rabbits were injected with two antigens—penicillinase and human albumin—diluted in saline and blended homogeneously into Freund's adjuvant. All animals in this group were given one intramuscular injection of a solution composed of 300,000 units of penicillinase and 10 mg. of human albumin in 0.5 ml. of saline blended with 1 ml. of Freund's adjuvant containing 0.5 mg. of *Mycobacterium butyricum* (Bacto-Adjuvant-Complete, Difco Laboratories, Detroit, Michigan).

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*The penicillinase for this work was supplied to us by Schenley Laboratories, Inc.

TABLE I

IMMUNOLOGIC PROPERTIES OF ANTISERA											
GROUP	GUINEA PIG NUMBER	SENSITIZED WITH	SKIN TESTING WITH PENICILLINASE		ANTISERA INDIVIDUALLY			ANTISERA POOLED (EQUAL VOLUMES OF EACH)			
			IMMEDIATE RESPONSE (b)	ARTHUS RESPONSE	PRECIPITATION (c)	PCA IN 1:5 DILUTION (d)	PRECIPITATION (TYTTER) (e)	AGGLUTINATION (TYTTER) (f)	PCA (TYTTER) (g)	PKR (g)	
I	1 (a)	Penicillinase									
	2	Human albumin	+++	Arthus	+++	+++					
	3	Adjuvant	+++	Arthus	++	+++					
	4		++	-	++	++					
	5		+++	Arthus	++	+++					
	6		+++	Arthus	++	+++					
	7		+	-	+	+++					
	8		++	-	+	+++					
	9 (a)					(1:80)	+	+	+	+	-
II	10	Penicillinase	+	-	+	++					
	11	Human albumin	-	-	+	+					
	12		+	-	+	++					
	13		-	-	+	++					
	14		+	-	+	++					
	15		-	-	+	+					
	16		+	-	+	++					
	17		-	-	+	++					
	18 (a)					(1:10)	+	+	+	+	-
III	19	Penicillinase									
	20	Adjuvant	+	-	+	+++					
	21		+++	Arthus	+++	+++					
	22		+++	Arthus	+++	+++					
	23		+	-	+	++					
	24		+++	Arthus	+++	+++					
	25		+++	Arthus	+++	+++					
	26		+++	Arthus	++	+++					
	27		+++	Arthus	+++	+++					

This injection was followed for a period of two weeks by semiweekly intramuscular administration of the same quantities of the two antigens in 0.5 ml. of saline blended with 0.5 ml. of Freund's adjuvant without mycobacteria (Bacto-Adjuvant-Incomplete, Difco Laboratories).

Group II: Nine guinea pigs and two rabbits were injected with two antigens, as in Group I, but without Freund's adjuvant.

Group III: Nine guinea pigs and two rabbits were injected with penicillinase only; 300,000 units of penicillinase in 0.5 ml. of saline were mixed with 1 ml. of Freund's adjuvant with mycobacteria. The initial injection was followed by semiweekly intramuscular administration of the same amount of penicillinase in 0.5 ml. of saline and 0.5 ml. of Freund's adjuvant without mycobacteria, over a period of two weeks.

Group IV: Nine guinea pigs and two rabbits were injected with penicillinase in saline (as in Group III) but without Freund's adjuvant.

Skin Testing.—Seven days before bleeding, all of the sensitized animals were tested by intradermal injection of 25,000 units of penicillinase in 0.1 ml. of saline. (Skin tests on normal animals failed to produce reactivity upon injection of the same and double the concentration of penicillinase.) The reactions were read several times within forty-eight hours after testing and were considered positive if, within the first hour, an erythema and edema developed at the tested sites. If this immediate reaction progressed to central blanching, infiltration, and central necrosis, the reactions were recorded forty-eight hours later as Arthus's phenomena.

Sera.—Three weeks after completion of the injection course, and seven days after skin testing, 10 ml. of blood was withdrawn from each animal and the sera were individually tested for precipitins and anaphylactic reactivity. Then equal volumes of the sera obtained from each of the animals were pooled by groups and the pooled sera were tested by quantitative immunologic procedures for precipitins, agglutinins, anaphylactic property (guinea pig smooth muscle-sensitizing property),⁶ and human-skin-sensitizing property (Table I).

*Passive Cutaneous Anaphylaxis in Guinea Pig (PCA)*⁶.—Male albino guinea pigs, weighing 350 grams each, were used. Shaved abdominal skin was employed for passive transfer of 0.1 ml. per site of antisera or antisera dilutions in 0.85 per cent saline. Six hours later an intracardial challenge with a mixture of 300,000 units of penicillinase and 1 ml. of 1 per cent Evans blue was given. The antiserum-saline dilution responsible for a minimal (7 mm. diameter) effusion of dye established the titration end point, that is, the maximal dilution of antibody capable of inducing a PCA reaction. The titration by dilution permitted the estimation of the sensitizing potency of the investigated sera and quantitative comparisons.

Passive Systemic Anaphylaxis (PSA).—Passive systemic sensitization of guinea pigs was accomplished by an intraperitoneal injection of 5 cm. of sensitive serum; twenty-four hours later the animals were challenged intravenously with 300,000 units of penicillinase. The PSA was considered positive if anaphylactic dyspnea ended in death and autopsy revealed emphysematous lungs.

Precipitation.—The technique described as agar gel diffusion Oudin⁷ and the classical test tube method were used. For the test tube method, sera were diluted twofold in series with saline, and an equal amount of antigen (100,000 units penicillinase in 0.1 ml.) was added to each test tube. The mixture was incubated for two hours at 37° C. and at 5° C. overnight. Results were read the following day.

Agglutination.—To increasing dilutions of serum was added an equal volume of a suspension of washed *Bacillus cereus*, grown on Bacto-brain-heart infusion broth and dextrose medium containing 10 international units of penicillin per one milliliter.⁸ The mixture was incubated for two hours at 37° C. and at room temperature overnight. Results were read the following day.

Prausnitz-Küstner Reactions (PKR).—Seitz-filtered sera, undiluted and diluted in 0.85 per cent saline, were injected intracutaneously in a volume of 0.1 ml. in normal subjects. The skin sites were challenged with 2,500 units of penicillinase in 0.05 ml. of saline forty-eight hours later.

Controls.—Parallel tests with normal rabbit and guinea pig sera served as controls. These were negative except that agglutination of *Bacillus cereus* occurred with both normal guinea pig and normal rabbit serum in dilutions through 1:10 but not higher.

RESULTS AND DISCUSSION

Table I shows the result of an attempt to sensitize thirty-six guinea pigs with penicillinase. All animals became sensitive. This is demonstrated by various immunologic tests. The Freund adjuvant markedly potentiated the sensitization as shown by the results of skin testing and by the increase in antipenicillinase precipitins, agglutinins, and anaphylactic reactivity (PCA). Although seven out of sixteen guinea pigs injected without adjuvant failed to reveal any skin reaction, antipenicillinase precipitins (determined by the agar gel diffusion technique) and anaphylactic reactivity (determined by PCA and PCA end point titrations) were noted in their sera.

All the antisera of the guinea pigs which were injected with both penicillinase and human albumin contained precipitins and anaphylactic antibody against both antigens. Pooled antisera of these animals induced PCA reactivity to albumin in dilutions 1:1,600 (Group I) and 1:100 (Group II).

Eight rabbits injected by the same technique as guinea pigs became sensitive to penicillinase as judged by tests for precipitins, agglutinins, anaphylactic reactivity, and human-skin-sensitizing property. The titer of antibodies in the rabbits' pooled sera was found to be higher than in the guinea pigs' pooled sera. (For instance, the PCA titers in the rabbits' pooled sera was as high as 1:8,000 for Group I, 1:1,000 for Group II, 1:16,000 for Group III, and 1:1,000 for Group IV).

Contrary to the negative results of passive sensitization of human skin obtained with guinea pig antisera, the sera of two rabbits transferred sensitivity to human skin in dilutions of 1:40 and 1:20. The capacity of these two

sera to induce human skin sensitivity was not proportional to their capacity to induce PCA, precipitation, and agglutination (as reflected by PCA, precipitation, and agglutination titers).

SUMMARY

Thirty-six guinea pigs and nine rabbits were given five semiweekly injections of penicillinase. Some animals received pure penicillinase, whereas others received it in combination with human albumin and/or Freund's adjuvant. All of the animals became sensitive to penicillinase. The degree of sensitivity was measured quantitatively by determining levels of circulating precipitins, agglutinins, and anaphylactic reactivity. Freund's adjuvant potentiated the sensitizing activity of penicillinase.

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