Passive Cutaneous Anaphylaxis in the Guinea Pig as Related to the Response of Isolated Ileum. (26227)

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Passive cutaneous anaphylaxis (PCA) has become in recent years a frequently utilized tool in investigation of various immunological problems, such as detection of small amounts of antibody or antigen (reviewed in 1), competitive fixation of normal gamma-globulins* and antibodies(2), the mechanism of reversed passive anaphylaxis(3), demonstration of auto-antibodies(4,5), differentiation between isophile and heterophile antibodies to sheep erythrocytes(6), study of the role of complement in anaphylaxis(7), the reaction of systemic lupus erythematosus with DNA (8), study of antibody-hapten interactions *in vivo*(9) and others.

In fact, the easily performed PCA technic (Ovary test) tends to replace the more elaborate Schultz-Dale test, usually regarded as the most reliable procedure for quantitative assay of anaphylactic sensitization. It was therefore decided to investigate the relative merits of both tests in parallel experiments with guinea pigs sensitized by known amounts of antibody or antigen. The investigation has confirmed the reliability of the PCA technic both in direct and reversed passive anaphylaxis.

Materials and methods. Albino guinea pigs of 200 ± 20 g were used throughout the experiments. The antigens used were: a) for direct passive anaphylaxis, chicken egg albumin (Ea) 3 times crystallized, prepared according to the method of Kekwick & Cannan (cf. 11, p 448); b) for reversed passive anaphylaxis, rabbit gamma globulin (RGG) as prepared by Kendall (cf. 11, p. 461).

Guinea pigs were immunized against RGG with the aid of Freund's adjuvant. The pooled guinea pig antisera titrated 0.03 mg AbN per ml. Anti-Ea was prepared in rabbits and titrated 0.400 mg AbN per ml.

Ovary test. In direct PCA, amounts of anti-Ea ranging from 10 to 100 mcg AbN were injected intravenously and followed at intervals of 2, 6, and 24 hours, by intradermal injection of 0.1 ml of adequate dilutions of Ea. In reversed PCA experiments, 0.500 mg RGG were injected intravenously followed 2-120 hours later by 0.1 ml of adequate dilutions of guinea pig anti-RGG. In either case, intradermal injections were immediately preceded by intravenous injection of 0.5 ml of 1% Geigy Blue in saline. Animals were sacrificed 15 minutes after intradermal challenge and the diameter of the reaction was measured on the internal side of the skin.†

Schultz-Dale test, Ileum segments of guinea pigs injected intravenously with anti-Ea or RGG, as indicated above, were tested in 10 ml of Tyrode's solution in a smooth muscle bath. 0.1 ml of adequate dilutions of Ea or anti-RGG was added to the bath and the intensity of contraction was compared to that provoked by addition of 0.2 ml of a 1:5000.000 dilution of histamine expressed as base. Results were recorded as +, ± or 0, a + result corresponding to a contraction equal or greater than elicited by the histamine standard.

Results. The results obtained are recorded in Tables I and II.

Discussion. In guinea pigs sensitized by intravenous injection of antigen (RGG) or antibody (rabbit anti-Ea) and challenged with the other reactant after adequate latency periods, a reasonable degree of parallelism was found between PCA response and

* The ability of normal rabbit gamma-globulin to inhibit PCA reactions to rabbit antibody (Ovary and Bier (2)) led us to investigate the possibility of producing reversed PCA by using rabbit gamma-globulin as an antigen (Bier and Siqueira(3)). This was done without knowledge of the work of Van den Ende(10), which only came to our attention after paper(3) was published.

† In some experiments results were recorded as ± (less than 5 mm), + (5-10 mm), ++ (10-15 mm), and +++ (15-20 mm).

TABLE I. A Comparison of Ovary and Schultz-Dale Tests in Direct Passive Anaphylaxis. Guinea pigs injected intravenously with varying amounts of rabbit anti-Ea and challenged with Ea after different latency periods (direct passive anaphylaxis).

Anti-Ea N intrav., μg	Latency period, hr	Ovary test with Ea, intraderm. inj. EaN, μg			Schultz-Dale test after addition of EaN, μg			
		5	.5	.05	50	5	.5	.05
100	2	25	14	10	+	+	—	—
		23	10	0	+	+	+	+
		10	0	0	+	+	+	±
		0	0	0	+	+	+	+
	6	17	10	15	+	+	+	+
		20	21	12	+	+	+	+
		27	20	18	+	+	+	+
		25	22	15	+	+	+	+
	24	28	23	15	+	+	+	—
		25	17	12	+	+	+	+
		30	19	15	+	+	+	+
		25	19	14	+	+	+	+
50	2	21	17	13	+	+	—	—
		0	0	0	—	—	—	—
		10	0	0	+	+	—	—
			10	0	+	+	—	—
	6	18	12	10	+	+	+	—
		21	20	17	+	+	—	—
		13	11	8	+	+	+	—
		20	16	14	+	+	+	—
	24	25	16	11	+	+	+	—
		24	18	12	+	+	+	—
		24	19	13	—	—	—	—
		26	25	14	+	+	+	—
25	2	14	8	7	+	—	—	—
		20	8	0	+	—	—	—
		22	9	9	+	+	—	—
		25	15	13	+	+	+	—
	6	20	15	10	+	+	+	+
		18	13	10	+	+	+	—
		26	20	17	+	+	+	+
		10	6	6	+	+	+	—
	24	27	21	17	+	+	+	—
		20	14	19	+	+	+	—
		32	18	16	+	+	+	—
		22	16	12	+	+	+	±
10	2	14	11	0	—	—	—	—
		14	10	0	—	—	—	—
		0	10	0	—	—	—	—
		0	10	0	—	—	—	—
	6	14	7	0	+	—	—	—
		20	13	10	+	+	—	—
		21	12	11	+	+	—	—
		16	14	5	+	+	—	—
	24	20	11	11	+	—	—	—
		18	10	10	+	+	+	+
		27	16	13	+	+	—	—
		22	15	14	+	+	+	—
Controls not inj. with anti-Ea	—	—	—	—				
		—	—	—				
		—	—	—				
		—	—	—				
		—	—	—				
		—	—	—				

TABLE II. A Comparison of Ovary and Schultz-Dale Tests in Reversed Passive Anaphylaxis. Guinea pigs injected intravenously with 0.5 mg RGG N and challenged with anti-RGG after different latency periods.

Latency period, hr	Ovary test with intraderm. inj. anti-RGG: 1:50	1:100	1:200	1:500	Schultz-Dale test after addition of anti-RGG: 1:1	1:10
2		±	0	0	0	0
		±	±	0	0	0
		±	±	0	0	0
		+	+	0	0	0
		+	±	0	+	0
4		++	+	±		
		++	++	++		
		+	+	+		
		++	++	±		
6		+	+	±	+	+
		++	+	±	+	+
		++	+	±	+	+
		++	+	0	+	0
8		++	++	±	+	0
		++	+	±		
		+++	+++	+++		
		+++	+++	++		
24	+++			+	+	+
	++			±	+	+
	++			±	+	+
					+	0
48	+++			++	0	0
	++			+	0	0
	++			±	+	0
					0	0
					0	0
					+	0
					+	0
					+	0
72			++		+	0
			++		+	+
			+		+	0
					0	0
96		±	0		0	0
		+++	+++		0	0
		++	+		0	0
		+++	++		+	+
120		++	+			
		++	±			
		±	±			
Controls not inj. with RGG		—	—	—		
		±	±	—		
		—	—	—		
		—	—	—		
		±	—	—		
	±			—		
	±			—		
	—			—		
	—			—		

the classical Schultz-Dale test (Tables I and II). This finding seems to indicate that in passive reverse anaphylaxis when the intravenous route is used for sensitization a common mechanism is involved which accounts for the local accumulation of dye and for the contraction of smooth muscle.

As pointed out by *Ovary*(3b), the severe and sometimes fatal reaction observed after successive intravenous injections of antigen and antibody may be ascribed to the action of toxic antigen-antibody complexes and eventually to the formation of anaphylatoxin. While these cases may not really correspond to passive reverse anaphylaxis, experiments such as recorded in Table II indicate that previous fixation of the gamma globulin antigen is an essential prerequisite for sensitization, as pointed out by *Van den Ende* and recently confirmed by others (3a, 3b, 3c, 12).

In the present experiments, dye accumulation after intradermal challenge proved to be a more sensitive test for detecting sensitization after too short or too long latency periods, particularly when a small amount of antibody was used, as shown in Table I for 10 μg AbN and a latency period of 2 hours, and in Table II for a latency period of 96 hours. Under these circumstances, minimal amounts of either antibody or antigen are revealed by PCA which would be undetected or only irregularly detected by the more cumbersome Schultz-Dale technic.

Summary. Guinea pigs were sensitized by intravenous injection of known amounts of antibody or antigen and challenged after varying latency periods with the other reactant. A comparison of the results obtained by local accumulation of dye after intradermal challenge (Ovary test) and by the classical Schultz-Dale test showed that the former behaves as a more sensitive test, particularly when small amounts of antibodies or antigen are involved and after inadequate latency periods. It is therefore justifiable to use Ovary's test as a reliable substitute for the Schultz-Dale technic in qualitative and quantitative assays of anaphylactic sensitization.

1. Ovary, Z., in: *Progress in Allergy,* v5, p459. Edited by P. Kallós, S. Karger, Basel, New York, 1958.

2. Ovary, Z., Bier, O. G., *Ann. Inst. Pasteur,* 1953, v84, 443; Prochazka Fisher, J., Cooke, R. A., *J.*

Allergy, 1957, v28, 150; Biozzi, G., Halpern, B. N., Binaghi, R., *J. Immunol.,* 1959, v82, 215.

3. (a) Bier, O. G., Siqueira, M., *Int. Arch. Allergy,* 1955, v6, 391; (b) Ovary, Z., *Immunology,* 1960, v3, 19; (c) Maurer, P. H., Thorpe, R. M., *J. Immunol.,* 1960, v84, 318.

4. Voisin, G. A., *Rev. Hematol.,* 1956, v11, 49.

5. Ovary, Z., Randall, H., Witebsky, E., Rose, N. R., Schulman, S. Metzgar, R., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 397.

6. Bier, O. G., Siqueira, M., Ovary, Z., *Abstr. Congr. Int. Microbiol.,* Rome, v1, 438; Ovary, Z., *Acta Allergol.* 1953, Suppl. III, 170.

7. (a) Bier, O. G., Siqueira, M., Osler, A. G., *Int. Arch. Allergy,* 1955, v7, 1; (b) Osler, A. G., Hawrisiak, M. M., Ovary, Z., Siqueira, M., Bier, O. G., *J. Exp. Med.,* 1957, v106, 811.

8. Deicher, H. R. G., Holman, H. R., Kunkel, H. G., Ovary, Z., *J. Immunol.,* 1960, v84, 106.

9. Ovary, Z., Karush, F., *ibid.,* 1960, v84, 409.

10. Van den Ende, M., *J. Hyg.,* 1940, v40, 377.

11. Kabat, E. A., Mayer, M. M., *Experimental Immunochemistry,* 1st ed., Chas. C Thomas, Springfield, Ill., 1948.

12. Humphrey, J. H., Mota, I., *Immunology,* 1959, v2, 19.

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Dephosphorylation of Psilocybin to Psilocin by Alkaline Phosphatase. (26228)

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A number of indolic compounds have gained pharmacological and biochemical interest because of their ability to produce psychotic states in human subjects. Thus, compounds such as bufotenin, N, N-dimethyltryptamine and harmine(1) have been reported to produce behavioural changes in man and various central nervous system effects in experimental animals. The most recent additions to this class of compounds are the substances found in the *Psilocybe mexicana* Heim mushroom, psilocybin and psilocin. Chemically, these compounds are 4-phosphoryl-N, N-dimethyltryptamine and 4-hydroxy-N, N-dimethyltryptamine, respectively. Their chemical structures are shown below.

According to Troxler *et al.*(2) psilocybin and psilocin are the first indole compounds of natural sources possessing substitutions on the 4-position of the indole ring. Psilocin differs from bufotenin only by having its –OH group located on the 4- rather than the 5-position.

The present report is concerned with hydrolysis of the phosphate group of psilocybin *in vitro* by a purified preparation of calf intestinal phosphatase. These studies were initiated because of the possibility that psilocybin might serve as a substrate for the phosphatase enzyme. This appeared reasonable since other aromatic compounds with a phosphate group are excellent substrates of acid and alkaline phosphatases(3).

Methods. All experiments in this study were carried out using purified calf intestinal phosphatase containing 15,000 units per mg.* Psilocybin and psilocin were generously supplied by Mr. Harry Althouse of Sandoz Pharmaceuticals, San Francisco, Calif.

Inorganic phosphate was determined according to a modified method of Fiske and Subbarrow(4). Psilocin was determined by adapting the colorimetric analysis for serotonin as described by Udenfriend *et al.*(5). All steps in the procedure were similar to that for serotonin. The final reaction between the nitrosonaphthol reagent and psilocin in an acid medium resulted in formation of a brownish-orange color as compared to the bright violet color of serotonin or bufotenin. The color with psilocin exhibited maximum absorption at wave lengths of 540 and at 430 mμ as read on the spectrophotometer. Determinations of psilocin were made at 430

* Purified alkaline phosphatase preparation was purchased from Mann Research Labs., New York.