

The heterogeneity of human antipenicillin skin-sensitizing antibodies

Statistical analysis of wheal responses elicited with benzylpenicillin, various penicilloyl haptenes, and their related compounds

Koji Shibata, M.D., Tsuyoshi Atsumi, M.D., and
Yoshihiko Horiuchi, M.D., Sapporo, Japan

Forty-six subjects who gave positive cutaneous reactions to benzylpenicilloyl-human serum albumin (BPO-HSA) or benzylpenicillin (Pc G) were further skin-tested with 14 kinds of test materials: benzylpenicillin, benzylpenicilloyl-epsilon-aminocaproate (BPO-EACA), divalent BPO-hexamethylenediamine, polyvalent BPO-human serum albumin conjugates, polyvalent BPO-poly-L-lysine conjugates, polyvalent penicilloyl-HSA conjugates of synthetic penicillins, polyvalent 6-aminopenicillanic acid-HSA conjugate, polyvalent cephalothin-HSA conjugate, and polyvalent conjugates of the acyl side-chain compounds of penicillins. Correlation coefficients among the diameters of wheals elicited with these test materials varied with each test antigen. In order to evaluate the heterogeneity of combining sites of the human skin-sensitizing antibodies, "factor analysis" was carried out. Eight independent factors were elucidated, which suggests that there are at least 8 different kinds of antibody molecules with respect to their binding properties for the different penicillin compounds.

Recently, benzylpenicilloyl (BPO) haptenic groups have been generally considered to be a major antigenic determinant responsible for human penicillin hypersensitivity.¹⁻⁴ In some clinical studies, however, it also was demonstrated that skin-testing with benzylpenicillin (Pc G) is a more specific index of immediate systemic reactions than testing with benzylpenicilloyl-polylysine.⁵⁻⁷ Thiel and colleagues⁸ further demonstrated that penicillenate, penilloate, penamaldoyl, or derivatives of 6-aminopenicillanic acid (6-APA) would frequently be potent inhibitors of hemagglutination reactions mediated by sera of subjects sensitive to benzylpenicillin. These findings indicate that Pc G

From the Department of Internal Medicine, Hokkaido University School of Medicine, Sapporo, Japan.

Received for publication Aug. 8, 1966.

will produce heterogeneous populations of antibody molecules, which seems to reflect the multiple metabolic pathways of Pe G *in vivo*. Similarly, Schlossman and Kabat⁹ and Gelzer and Kabat¹⁰ reported that the human antidextran antibodies consist of a heterogeneous population of molecules with different binding properties. This concept was also indicated by Kitagawa and colleagues¹¹⁻¹³ and Kreiter and colleagues¹⁴ by their studies of rabbit anti-p-azobenzenearsonate antibodies. However, by the usual immunological methods, such as direct skin tests and Prausnitz-Küstner reactions, it has been quite difficult to evaluate the heterogeneity of human skin-sensitizing antibodies.

The present study was designed to investigate the heterogeneity of combining sites of human skin-sensitizing antibodies to penicillin by "factor analysis" of wheal diameters evoked with Pe G and its related compounds.

MATERIALS AND METHODS

Test reagents*

Benzylpenicilloyl- ϵ -aminocaproate (BPO-EACA), benzylpenicilloyl-hexamethylenediamine (BPO₂-HMD), benzylpenicilloyl-polylysine (BPO-PLL), and penicilloyl-HSA conjugates of synthetic penicillins were prepared by modification of Levine's methods.^{15, 17} Cephalothin-HSA (CET-HSA) and 6-aminopenicillanic acid-HSA (6-APA-HSA) conjugates were prepared in the same manner as the preparation of penicilloyl-HSA conjugates. Phenylacetyl-glycyl-HSA (PAG-HSA) and 2,6-dimethoxybenzoylglycyl-HSA (DMBG-HSA) were prepared as described previously.¹⁶ All these skin-test reagents were analyzed quantitatively as described in the previous article¹⁶ and sterilized by filtration through HA Millipore membranes.

Skin-testing

A total of 2,242 subjects admitted in six hospitals in Sapporo and Otaru districts, Hokkaido, were skin-tested with each of the following four solutions: 3×10^{-6} M (BPO)₁₄-HSA, 3×10^{-6} M (PAG)₁₈-HSA, 500 units per milliliter of Pe G (each was dissolved in physiologic saline), and physiologic saline as a control. The intracutaneous injections were performed with 0.02 ml. of each antigen solution. The test sites were examined 15 minutes after injection for wheal reactions. Reaction intensities were recorded in millimeters as an average diameter. Wheals of greater than 6 mm. with pseudopods were considered positive. With (BPO)₁₄-HSA, 66 subjects (2.9 per cent) gave positive wheal responses. Three subjects were reactive only to Pe G. Of these 69 subjects, 46 (44 reactive to (BPO)₁₄-HSA and 2 reactive to Pe G) were further skin-tested with 14 kinds of antigens simultaneously at the sites chosen at random on their backs.

*Human serum albumin (HSA) and L-polylysine (molecular weight, 70,000) were purchased from Mann Research Laboratories, New York. Benzylpenicillin (Pe G), phenoxymethylpenicillin (Pe V), 2,6-dimethoxyphenylpenicillin (DMP Pe), 5-methyl-3-phenyl-4-isoxazolylpenicillin (MPI Pe), and 6-aminopenicillanic acid (6-APA) were donated by the Banyu Pharmaceutical Co., Tokyo. Cephalothin (CET) was donated by the Shionogi Pharmaceutical Co., Osaka.

Factor analysis¹⁸⁻²¹

Factor analysis is a method to reveal the structure of a phenomenon, which might be based on effects from several factors independent of one another.*

RESULTS

Skin responses

The wheal diameters evoked with 14 kinds of antigens in 46 subjects are listed in Table I. Univalent haptene (i.e., BPO-EACA) was found to elicit merely faint reactions in 3 subjects (Nos. 19, 22, and 30). Two of the 3 subjects who gave positive skin tests to Pe G had histories of anaphylactoid reactions to Pe G. Divalent haptene [i.e., (BPO)₂-HMD] and polyvalent (from 8 to 345) haptenes were almost equally effective in evoking cutaneous reactions. In contrast, most of wheal diameters elicited with penicilloyl-HSA conjugates of synthetic penicillins and their related compounds were smaller than those elicited with BPO haptenes. However, it is noteworthy that patterns of cutaneous reactions to these antigens were quite different from subject to subject. For example, Subject 29 showed strong positive reactions to BPO-PLL, (PAG)₁₈-HSA, and to phenoxymethylpenicilloyl-HSA [(PMPO)₁₅-HSA] conjugate, while negative results were obtained with the other antigens. In Subject 33, on the other hand, all the antigens tested except BPO-EACA, (DMBG)₁₅-HSA, and Pe G were effective elicitors of cutaneous reactions.

Table II shows the matrix of correlation coefficients among 14 kinds of test antigens. Pe G and BPO-EACA were not correlated with other testing agents, while the other 12 antigens were correlated with each other in various degrees. There were high correlations among (BPO)₈-PLL, (BPO)₁₄-HSA, and (BPO)₁₁₄-PLL. Correlation coefficients among other antigens were smaller than those among these three. The findings that the correlation coefficients varied from antigen to antigen and that there were many antigens with low correlation coefficients seem to suggest that skin-sensitizing antibodies to these antigens in a reaginic serum consist of populations of antibody molecules heterogeneous with respect to their binding properties.

*If Z_{ji} is the result of the i -th test in the j -th case, its structure will be given by the equation

$$Z_{ji} = a_{1j}F_{1i} + a_{2j}F_{2i} + \dots + a_{mj}F_{mi}$$

where F_{mi} ($m = 1, 2, \dots, m$) is the factor score for the m -th factor in the j -th case and a_{mj} is the factor load of F_m to the j -th test, corresponding to the correlation coefficient between Z_{ji} and F_{mi} . If $a_1 = 1$, the test Z_j is the factor F itself. Factors are defined to be mutually independent. From the pattern of factor load, the characteristics of the factor will be estimated. The validity of such a structure will be criticized by result itself. This method is used to obtain a hypothesis on a reasonable number of factors, which have to be confirmed experimentally. The schedule of analysis followed in the article was as follows: (1) setting a histogram; (2) transformation to normal distribution; (3) calculation of the matrix of correlation coefficients; (4) principal component analysis; (5) maximum likelihood analysis; (6) varimax analysis; (7) oblimax analysis; (8) calculation of angles between common factors by oblimax method; (9) calculation of factor scores with each individual.

Table I. *Wheal diameters (millimeters) evoked with 14 kinds of antigens in 46 subjects*

<i>Case</i>						
	<i>BPO-EACA</i>	<i>Pc G</i>	<i>(PAG)₁₈⁻</i> <i>HSA</i>	<i>(DMBG)₁₅⁻</i> <i>HSA</i>	<i>(CET)₂₆⁻</i> <i>HSA</i>	<i>(BPO)₅⁻</i> <i>PLL</i>
1	Neg.	Neg.	7.5	6.0	7.0	18.0
2	Neg.	Neg.	9.5	Neg.	9.0	24.0
3	Neg.	Neg.	7.5	Neg.	9.0	13.0
4	Neg.	Neg.	Neg.	Neg.	Neg.	10.5
5	Neg.	Neg.	7.0	Neg.	10.0	14.0
6	Neg.	Neg.	Neg.	Neg.	8.5	8.5
7	Neg.	Neg.	8.0	Neg.	6.0	9.0
8	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
9	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
10	Neg.	13.0	Neg.	Neg.	Neg.	Neg.
11	Neg.	Neg.	12.0	6.5	6.0	33.5
12	Neg.	Neg.	Neg.	Neg.	Neg.	10.0
13	Neg.	Neg.	8.5	Neg.	Neg.	13.0
14	Neg.	Neg.	11.0	Neg.	Neg.	12.0
15	Neg.	Neg.	6.0	Neg.	6.5	Neg.
16	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
17	Neg.	11.5	Neg.	Neg.	Neg.	Neg.
18	Neg.	Neg.	56.5	38.5	42.0	65.5
19	6.5	Neg.	6.5	Neg.	7.0	6.0
20	Neg.	Neg.	10.0	Neg.	Neg.	12.5
21	Neg.	Neg.	9.0	9.0	7.0	11.5
22	8.0	Neg.	7.0	Neg.	Neg.	26.0
23	Neg.	Neg.	12.0	6.0	8.0	20.0
24	Neg.	Neg.	7.0	Neg.	Neg.	11.0
25	Neg.	Neg.	Neg.	Neg.	Neg.	6.0
26	Neg.	Neg.	Neg.	Neg.	Neg.	8.5
27	Neg.	Neg.	Neg.	Neg.	7.0	8.0
28	Neg.	Neg.	7.0	Neg.	Neg.	14.5
29	Neg.	Neg.	13.0	Neg.	Neg.	15.5
30	9.0	Neg.	8.0	Neg.	Neg.	Neg.
31	Neg.	Neg.	13.0	13.0	22.5	23.0
32	Neg.	Neg.	17.0	10.5	11.5	24.5
33	Neg.	Neg.	14.5	Neg.	10.0	15.0
34	Neg.	Neg.	Neg.	Neg.	Neg.	9.5
35	Neg.	Neg.	Neg.	Neg.	Neg.	7.0
36	Neg.	Neg.	Neg.	Neg.	Neg.	6.5
37	Neg.	Neg.	10.0	7.5	8.5	12.5
38	Neg.	Neg.	11.5	Neg.	Neg.	20.0
39	Neg.	Neg.	7.5	Neg.	Neg.	11.5
40	Neg.	Neg.	11.0	Neg.	8.0	14.5
41	Neg.	Neg.	10.0	Neg.	Neg.	12.5
42	Neg.	Neg.	7.5	6.0	5.0	12.0
43	Neg.	Neg.	Neg.	Neg.	7.0	Neg.
44	Neg.	Neg.	Neg.	Neg.	Neg.	15.5
45	Neg.	Neg.	8.0	Neg.	Neg.	Neg.
46	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

<i>Antigen</i>							
$(BPO)_{14}$ HSA	$(BPO)_{14}$ PLL	$(BPO)_5$ HMD	$(BPO)_{315}$ PLL	$(6-APA)_{20}$ HSA	$(PMPO)_{15}$ HSA	$(DMPPQ)_{20}$ HSA	$(MPIPO)_{19}$ HSA
20.5	19.0	12.5	16.5	9.5	7.5	7.0	Neg.
31.5	14.0	19.0	23.0		13.5	21.5	19.0
9.5	11.0	12.0	14.5		8.0	6.5	Neg.
14.5	9.0	16.0	12.5		14.0	Neg.	Neg.
25.0	17.5	11.0	Neg.	8.0	23.0	11.0	14.5
7.5	Neg.	15.5	10.0	7.5	6.0	Neg.	Neg.
17.5	13.5	10.0	14.0		13.0	Neg.	Neg.
7.5	Neg.	Neg.	16.0	Neg.	9.0	Neg.	9.0
7.5	Neg.	7.5	Neg.		Neg.	Neg.	Neg.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
30.0	29.0	27.0	24.5	12.0	25.5	14.0	17.0
10.5	19.5	13.5	25.5		6.5	Neg.	Neg.
19.5	Neg.	Neg.	6.0	7.5	Neg.	Neg.	Neg.
16.5	Neg.	Neg.	9.0	Neg.	Neg.	Neg.	Neg.
6.5	6.5	10.0	10.5		Neg.	Neg.	Neg.
8.5	Neg.	Neg.	Neg.		5.5	Neg.	Neg.
Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
56.0	75.0	35.5	52.5		12.5	30.0	28.0
13.0	6.5	13.0	15.5		10.5	Neg.	9.5
23.5	12.5	Neg.	15.0	Neg.	9.5	Neg.	Neg.
28.5	20.5	34.0	27.0	Neg.	13.0	10.0	8.5
32.0	42.5	8.5	28.5		10.0	8.0	Neg.
22.5	26.0	14.0	21.0	Neg.	11.5	8.0	11.0
18.0	9.0	10.0	10.5		Neg.	Neg.	Neg.
14.0	Neg.	10.0	9.5		8.0	Neg.	Neg.
14.5	12.0	Neg.	16.0	Neg.	Neg.	Neg.	Neg.
8.5	Neg.	6.5	11.5		25.0	Neg.	14.0
19.5	17.0	19.5	15.5	Neg.	Neg.	Neg.	Neg.
31.0	13.5	Neg.	18.0		20.5	Neg.	Neg.
8.0	Neg.	Neg.	9.5		9.0	Neg.	7.5
31.0	32.5	13.0	26.0		20.0	13.0	9.5
22.0	21.5	21.5	25.5		24.0	10.0	17.0
36.0	29.0	16.5	32.0		43.0	20.5	25.5
10.5	Neg.	10.0	18.5		Neg.	11.5	Neg.
12.5	Neg.	Neg.	15.5		8.5	Neg.	Neg.
10.0	13.5	Neg.	12.5		15.5	9.0	12.5
21.5	13.5	22.5	17.0	11.5	21.0	9.0	13.5
16.5	17.5	22.0	21.5	Neg.	19.0	Neg.	Neg.
14.5	15.5	16.5	24.0	6.0	10.5	Neg.	6.0
23.5	32.5	27.0	31.5	8.0	26.5	7.0	13.5
19.5	13.0	22.0	16.5	Neg.	13.0	Neg.	Neg.
14.5	14.0	19.0	17.0	11.5	11.5	6.0	9.5
7.5	Neg.	Neg.	14.0	6.0	Neg.	Neg.	Neg.
21.0	18.5	7.0	20.0	8.0	16.5	6.5	Neg.
9.0	Neg.	Neg.	Neg.	Neg.	7.0	Neg.	Neg.
13.0	Neg.	10.5	7.0	Neg.	Neg.	Neg.	Neg.

Table II. *The matrix of the correlation coefficients*

Antigen*	1	2	3	4	5	6
1	1.00	-0.04	0.06	-0.07	0.12	-0.24
2		1.00	-0.21	-0.13	-0.12	-0.27
3			1.00	0.60	0.56	0.68
4				1.00	0.71	0.53
5					1.00	0.48
6						1.00
7						
8						
9						
10						
11						
12						
13						
14						

*1, BPO-EACA; 2, Pc G; 3, (PAG)₁₈-HSA; 4, (DMBG)₁₅-HSA; 5, (CET)₂₆-HSA; 6, (6-APA)₂₀-HSA; 12, (PMPO)₁₅-HSA; 13, (DMPPPO)₂₀-HSA; 14, (MPIPO)₁₉-HSA.

FACTOR ANALYSIS

Eight factors were obtained by analysis of the matrix of correlation coefficients.* In this case, the sum of lambda/N of component output accounted for 92.2 per cent. The primary irritancy of the antigens and the difference of individual reactivity to the antigen are discounted as "error" in this procedure. Thus most of the sizes of wheal diameters elicited with each antigen were explained by these 8 factors.

Factor loadings of 14 kinds of antigens on 8 orthogonal axes were calculated by the varimax method (Table III) and those on 8 oblique axes by the oblimax method. Each factor was represented by each axis. A strong similarity was seen in both patterns. Furthermore, in the oblimax solution the angles among these 8 factors projected into the space were nearly 90 degrees, with the exception of the two between Factors 3 and 5 and between Factors 5 and 7 (Table IV). Therefore, it seems plausible that these 8 factors will be approximately independent of each other and that the reactivities of each antigen will be represented by the vectors on the axes.

As seen in Table III, when the magnitude of the factor loadings is compared, it is clear that Factor 1 and Factor 2 are characterized specifically by

*The number of independent factors was determined according to the following two points: (1) per cent of total original communality was nearly 100, (2) residual variance covalence matrix was set by means of the equation

$$X = V^{-1} (C - \hat{e}) = X_{ij} \quad (i, j = 1, 2, \dots, 14)$$
from standardized correlation matrix (C), estimated correlation matrix ($\hat{e}$), and error variance matrix (V). It was followed by chi square test given by the following equation

$$\chi^2 = \left\{ n - \frac{1}{6} (2p + 5) - \frac{2}{3} k \right\} \sum_{i,j} x_{ij} \times x_{ij}$$

where the degree of freedom computed by the formula: $\frac{1}{2} \{ (p - k)^2 - (p + k) \}$ was 7. Here, n is the sample size of mean diameter, p is the number of antigens, and k is the number of factors. The observed chi square was 10.92. As the value of chi square with this degree of freedom is (0.05) = 14.07, the observed value is not significant. Therefore, the maximum number of independent factors must be 8.

7	8	9	10	11	12	13	14
-0.14	-0.18	-0.21	-0.06	0.00	0.01	-0.11	0.09
-0.47	-0.19	-0.22	-0.30	-0.03	-0.17	-0.13	-0.08
0.71	0.65	0.46	0.48	0.09	0.45	0.52	0.49
0.48	0.54	0.48	0.43	0.34	0.32	0.63	0.55
0.43	0.51	0.55	0.40	0.58	0.45	0.68	0.67
0.84	0.80	0.60	0.70	0.38	0.54	0.64	0.41
1.00	0.75	0.53	0.66	0.26	0.56	0.61	0.44
	1.00	0.62	0.69	0.31	0.63	0.66	0.48
		1.00	0.55	0.34	0.44	0.52	0.43
			1.00	0.16	0.49	0.49	0.40
				1.00	0.41	0.57	0.43
					1.00	0.52	0.67
						1.00	0.72
							1.00

(BPO)₈-PLL; 7, (BPO)₁₄-HSA; 8, (BPO)₁₁₄-PLL; 9, (BPO)₂-HMD; 10, (BPO)₃₄₅-PLL; 11,

the reactions elicited with BPO-EACA and Pc G, respectively. In Factor 3, the factor loads of the side chain groups, i.e., (DMBG)₁₃-HSA, (CET)₂₆-HSA, and (PAG)₁₈-HSA, are higher than those of other antigens. Accordingly, the reactions elicited with these antigens would be characterized by this factor. In the same way, Factor 4 would be characterized by (BPO)₈-PLL, (BPO)₁₄-HSA, and (BPO)₁₁₄-PLL. Factors 5, 6, 7, and 8 would be characterized by (BPO)₂-HMD, (BPO)₃₄₅-PLL, (6-APA)₂₀-HSA (the nuclear portion of the penicilloyl molecule), and methylphenylisoxazolylpenicilloyl₁₂-HSA [(MPI-PO)₁₂-HSA], respectively.

With each of 46 subjects the factor scores of these 8 factors were calculated. The factor scores represent the weights of these 8 factors influencing the wheal diameters in each subject. When the scores of these 8 factors were compared in each subject, the factors having high scores above 1.0 varied significantly from subject to subject except Factors 1 and 2.

These results seem to show that the wheal reactions elicited with each test antigen are dependent upon the combination of two or more factors, the pattern of which varies from subject to subject.

DISCUSSION

Forty-six subjects reactive to BPO or to Pc G were skin-tested simultaneously with 14 kinds of antigens. By factor analysis of the wheal diameters evoked with each antigen, 8 independent factors were elucidated. Since primary irritancies of the antigens and the differences of individual reactivities to the antigens were discounted as "error" in the procedure of the analysis, it seems plausible that these 8 factors will be characterized by 8 populations of skin-sensitizing antibodies with different binding properties.

Factor 2 is characterized by an antibody specificity complementary to Pc G. Of interest is the fact that 2 of the 3 subjects who gave positive cutaneous reactions to Pc G had a history of immediate systemic reactions. Siegel and

Table III. *Factor matrix by the varimax method*

<i>Test materials</i>	<i>F 1</i>	<i>F 2</i>	<i>F 3</i>	<i>F 4</i>	<i>F 5</i>	<i>F 6</i>	<i>F 7</i>	<i>F 8</i>
BPO-EACA	0.99	-0.03	-0.03	-0.09	-0.07	-0.01	0.00	0.05
Pc G	-0.04	0.97	-0.03	-0.18	-0.06	-0.07	0.00	-0.02
(PAG) ₁₈ -HSA	0.14	-0.05	0.52	0.74	0.10	-0.08	-0.16	0.18
(DMBG) ₁₅ -HSA	-0.02	-0.03	0.62	0.36	0.14	0.10	0.12	0.23
(CET) ₂₆ -HSA	-0.08	-0.04	0.81	0.18	0.17	0.10	0.32	0.30
(BPO) ₈ -PLL	-0.16	-0.10	0.17	0.82	0.16	0.22	0.22	0.16
(BPO) ₁₄ -HSA	-0.08	-0.32	0.16	0.80	0.10	0.14	0.09	0.24
(BPO) ₁₁₄ -PLL	-0.11	-0.02	0.23	0.70	0.24	0.26	0.12	0.27
(BPO) ₂ -HMD	-0.12	-0.10	0.27	0.35	0.82	0.14	0.16	0.19
(BPO) ₈₄₅ -PLL	0.00	-0.14	0.16	0.52	0.20	0.71	0.00	0.21
(6-APA) ₂₀ -HSA	0.01	0.00	0.22	0.10	0.10	0.00	0.93	0.23
(PMPO) ₁₅ -HSA	0.03	-0.06	0.11	0.42	0.13	0.14	0.22	0.58
(DMPPPO) ₂₀ -HSA	-0.08	-0.02	0.38	0.42	0.13	0.12	0.34	0.50
(MPIPO) ₁₉ -HSA	0.08	-0.01	0.41	0.18	0.10	0.07	0.14	0.83

Table IV. *Angles between common factors (degree) by the oblimax method*

	<i>F 1</i>	<i>F 2</i>	<i>F 3</i>	<i>F 4</i>	<i>F 5</i>	<i>F 6</i>	<i>F 7</i>	<i>F 8</i>
F 1	0.0	87.5	71.2	-84.5	-76.3	80.3	76.2	-89.2
F 2		0.0	77.0	-78.5	85.2	87.4	-71.2	87.3
F 3			0.0	85.4	52.2	-85.3	73.2	-88.3
F 4				0.0	82.3	82.4	85.5	-88.3
F 5					0.0	89.1	62.2	-87.3
F 6						0.0	72.0	-77.1
F 7							0.0	-86.5
F 8								0.0

Levine⁵ reported that 4 of 5 sera from immediate systemic reactors gave positive passive transfer reactions to Pc G but did not react to any of the polyvalent conjugates containing BPO, BPE, and penicillamine haptenic groups.

Factor 1 is characterized by the reaction evoked with BPO-EACA. The univalent hapten elicited merely a faint reaction in 3 subjects. Therefore, the meaning of the cutaneous reaction evoked with a univalent hapten remains to be studied.

The other six factors seem to be characterized by heterogeneous populations of skin-sensitizing antibodies which are cross-reactive with each other. The absorption test has been generally employed to detect cross-reacting skin-sensitizing antibodies coexisting in a reaginic serum. However, when a number of heterogeneous populations of skin-sensitizing antibodies coexist in a reaginic serum and the constitutional pattern of the antibodies varies from subject to subject, it would be difficult to evaluate the heterogeneity quantitatively. In this case, the intensity of a wheal-and-flare reaction evoked with an antigen would be interpreted as the sum of antigen-antibody reactions between the antigen and its homologous antibody and between the antigen and the cross-reacting antibodies. Accordingly, if wheal diameters evoked with an antigen in sufficient numbers of subjects are treated by factor analysis and if several kinds of independent factors are elucidated as a result, the factors seem to be

characterized by skin-sensitizing antibodies with different combining sites. For example, if a reaginic serum contains two antibody populations and one is complementary to the entire BPO molecule and the other is complementary to 6-APA (the nuclear portion of the BPO molecule), the polyvalent BPO hapten would react more intensely with the former than the latter. And if the constitutional ratio of the two antibody populations varies from subject to subject, two independent factors which represent each antibody specificity must be elucidated by factor analysis of the wheals.

In fact, an antibody specificity complementary to (6-APA)₂₀-HSA, which corresponds to the nuclear portion of the BPO molecule, was elucidated as Factor 7. In a study of antibody specificities of individual sera from penicillin allergy, Thiel and colleagues⁸ demonstrated by their hemagglutination inhibition experiments that among a variety of specific inhibition patterns there was an antibody specificity for which 6-APA or its polymer was the best inhibitor.

Factor 3 has a large factor load on the acyl side-chain groups of the penicillin molecule. It is characterized by an antibody complementary to (PAG)₁₈-HSA, (DMBG)₁₃-HSA, and (CET)₂₆-HSA. This fact seems to be consistent with the demonstration, as described in another²⁴ of our articles, that cephalothin (CET) had a strong cross-reactivity with rabbit anti-BPO antibody, which would be attributed mainly to the stereochemical similarity (i.e., aromaticity) of the side-chain portion of Pc G and CET. Another²⁵ of our articles demonstrated that the rabbit anti-BPO antibody produced in a single rabbit could be separated on a specific immunoabsorbent by elution with suitable haptens into some fractions complementary to the acyl side-chain portion, to the nuclear portion and to the entire BPO molecule containing carrier proteins. From this experiment it seems reasonable to speculate that human anti-BPO skin-sensitizing antibody would also consist of some antibody populations having different combining sites adapted to different parts of the BPO molecule.

Factor 8 has a large factor load on penicilloyl conjugates of synthetic penicillins, especially of MPIPe. This seems to demonstrate that Factor 8 would correspond to the antibodies specific for penicilloyl haptens of synthetic penicillins, in view of prevalent usage of many synthetic penicillins. The authors had reported¹⁶ that some of the specific rabbit antibodies to penicilloyl-protein conjugates of synthetic penicillins were highly cross-reactive with penicilloyl conjugates of other penicillins.

From the value of factor loads, it seems plausible that Factors 4, 5, and 6 will correspond to the antibody adapted to BPO haptens. The difference among these three factors consists in the difference of numbers of the BPO groups contained per mole conjugate. Of interest is that Factors 5 and 6 are distinguished from Factor 4. Factor 4 corresponds to the antibody specificity adapted to the polyvalent hapten containing 8 to 114 BPO groups per mole conjugate. Factor 5 corresponds to the divalent hapten of (BPO)₂-HMD and Factor 6 to the polyvalent hapten in which 345 out of 550 ϵ -NH₂ groups of PLL are coupled with BPO groups. Levine²² has demonstrated that PCA

mediated by antibodies of comparatively high binding affinity can be elicited more effectively by the divalent hapten than by polyvalent haptens, whereas PCA mediated by antibodies of comparatively low binding affinity requires polyvalent haptens for effective elicitation. Levine²³ further demonstrated that the abilities of BPO conjugates to evoke wheal-and-flare reactions in BPO-sensitive subjects were dependent upon the numbers of BPO groups contained per mole conjugate. The reason why the antigenic reactivities of BPO haptens are different with the numbers of BPO groups contained per mole conjugate remains to be studied.

The results of factor analysis seem to suggest that at least 8 kinds of antibody specificities are involved in humans showing skin test reactions to penicillin antigens.

The authors wish to thank Professor Keimei Mashimo, Department of Medicine, Hokkaido University School of Medicine, for his critical review of this manuscript, and to thank Dr. Kosei Takahashi, Department of Physical Therapy and Medicine, University School of Tokyo, and Dr. Choichiro Asano, Shionogi Computer Center, for their helpful advice and statistical analysis.

REFERENCES

1. Levine, B. B.: Studies on the Mechanism of Formation of the Penicillin Antigen. I. Delayed Allergic Cross-Reactions Among Penicillin G and Its Degradation Products, *J. Exper. Med.* **112**: 1131, 1960.
2. Levine, B. B., and Ovary, Z.: Studies on the Mechanism of Formation of the Penicillin Antigen. III. The N-(D- α -Benzyl-penicilloyl) Group as an Antigenic Determinant Responsible for Hypersensitivity to Penicillin G, *J. Exper. Med.* **114**: 875, 1961.
3. Levine, B. B., and Price, V. H.: Studies on the Immunological Mechanisms of Penicillin Allergy. II. Antigenic Specificities of Allergic Wheal-and-Flare Skin Responses in Patients With Histories of Penicillin Allergy, *Immunology* **7**: 542, 1964.
4. Parker, C. W., Shapiro, J., Kern, M., and Eisen, H. N.: Hypersensitivity to Penicillenic Acid Derivatives in Human Beings With Penicillin Allergy, *J. Exper. Med.* **115**: 821, 1962.
5. Siegel, B. B., and Levine, B. B.: Antigenic Specificities of Skin-Sensitizing Antibodies in Sera From Patients With Immediate Systemic Allergic Reactions to Penicillin, *J. ALLERGY* **35**: 488, 1964.
6. Finke, S. R., Grieco, M. H., Connell, J. T., Smith, E. C., and Sherman, W. B.: Results of Comparative Skin Tests With Penicilloyl-Polylysine and Penicillin in Patients With Penicillin Allergy, *Am. J. Med.* **38**: 71, 1965.
7. Horiuchi, Y., Shibata, K., and Atsumi, T.: Immediate Type Allergic Skin Response With Various Penicilloyl Haptens, *Jap. J. Allergy* **15**: 474, 1966.
8. Thiel, A. J., Mitchell, S., and Parker, C. W.: The Specificity of Hemagglutination Reactions in Human and Experimental Penicillin Hypersensitivity, *J. ALLERGY* **35**: 399, 1965.
9. Schlossman, S. F., and Kabat, E. A.: Specific Fractionation of a Population of Antidextran Molecules With Combining Sites of Various Sizes, *J. Exper. Med.* **116**: 535, 1962.
10. Gelzer, J., and Kabat, E. A.: Specific Fractionation of Human Antidextran Antibodies. II. Assay of Human Antidextran Sera and Specifically Fractionated Purified Antibodies by Microcomplement Fixation and Complement Fixation Inhibition Techniques, *J. Exper. Med.* **119**, 983, 1964.
11. Kitagawa, M., Yagi, Y., and Pressman, D.: The Heterogeneity of Combining Sites of Antibodies as Determined by Specific Immunoabsorbents. I. A Rapid Micromethod of Fractionation of Antibody, *J. Immunol.* **95**: 446, 1965.
12. Kitagawa, M., Yagi, Y., and Pressman, D.: The Heterogeneity of Combining Sites of Antibodies as Determined by Specific Immunoabsorbents. II. Comparison of Elution Patterns With Anti-p-azobenzoate Antibodies by Different Kinds of Immunoabsorbent and Eluting Hapten, *J. Immunol.* **95**: 455, 1965.
13. Kitagawa, M., Yagi, Y., and Pressman, D.: The Heterogeneity of Combining Sites of Antibodies as Determined by Specific Immunoabsorbents. III. Further Characterization of Purified Antibody Fractions Obtained From Anti-p-azobenzoate Antibodies, *J. Immunol.* **95**: 991, 1965.
14. Kreiter, V. P., and Pressman, D.: Fractionation of Anti-p-azobenzene-arsenate Antibody by Means of Immunoabsorbents, *Immunochemistry* **1**: 91, 1964.

15. Levine, B. B.: N-(α -D-Penicilloyl)-Amines as Univalent Hapten Inhibitors of Antibody-Dependent Allergic Reactions to Penicillin, *J. M. & Pharm. Chem.* **5**: 1025, 1962.
16. Horiuchi, Y., and Shibata, K.: Immunological Studies on the Antigenic Bindings of Benzylpenicillin and Five Synthetic Penicillins With Proteins, *Internat. Arch. Allergy* **28**: 306, 1965.
17. Levine, B. B.: Studies on the Immunological Mechanisms of Penicillin Allergy. I. Antigenic Specificities of Guinea-Pig Skin Sensitizing Rabbit Anti-benzylpenicillin Antibodies, *Immunology* **7**: 527, 1964.
18. Harman, H. H.: *Modern Factor Analysis*, Chicago, 1960, University of Chicago Press.
19. Cooley, W. W., and Lohnes, P. R.: *Multivariate Procedure for the Behavioral Sciences*, New York, 1964, John Wiley & Sons, Inc.
20. Seal, H.: *Multivariate Statistical Analysis for Biologist*, London, 1965, Methuen & Company, Ltd.
21. Thurston, L. L.: *Multiple Factor Analysis*, Chicago, 1947, University of Chicago Press.
22. Levine, B. B.: The Nature of the Antigen-Antibody Complexes Which Initiate Anaphylactic Reactions. I. A Quantitative Comparison of the Abilities of Nontoxic Univalent, Toxic Univalent, Divalent and Multivalent Benzylpenicilloyl Haptens to Evoke Passive Cutaneous Anaphylaxis in the Guinea-Pig, *J. Immunol.* **94**: 111, 1965.
23. Levine, B. B., and Fellner, J.: The Nature of Immune Complexes Initiating Allergic Wheal-and-Flare Reactions, *J. ALLERGY* **36**: 342, 1965.
24. Shibata, K., Atsumi, T., Horiuchi, Y., and Mashimo, K.: Immunological Cross-Reactivities of Cephalothin and Its Related Compounds With Benzylpenicillin (Penicillin G), *Nature* **212**: 419, 1966.
25. Atsumi, T., Nishida, K., Kinoshita, Y., Shibata, K., and Horiuchi, Y.: The Heterogeneity of Combining Sites of Benzylpenicilloyl Antibodies Obtained From Single Rabbit: Fractionation of Antibodies With Specific Immunoabsorbent, *J. Immunol.* **99**: 1286, 1967.