THE PRECIPITIN REACTION OF ANTIPNEUMOCOCCUS SERA.*

II. The Ratio of Precipitin to Protective Antibody

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In the diagnosis of infectious disease precipitin and agglutinin reactions have assisted in the identification of the invading organism, when the patient's serum has been tested with suspected organisms or when antigenic material from the patient (1) has been tested against antisera.

Agglutination has been used also in the quantitative determination of the antibody in antityphoid and antimeningococcus sera. For the standardization of diphtheria antitoxin Ramon (2; see also reference 3) suggested a test-tube method based on the formation of a flocculent precipitate when toxin and antitoxin were combined in definite proportions.

The present paper records investigations concerning the parallelism of the precipitin with the protective antibody in antipneumococcus sera. The quantitative determination of antibody in antipneumococcus sera is based on the relative reactivity of the precipitin and the protective antibody; the question of their identity is not raised.

Agglutination tests in pneumonia require the use of uniform cultures; they thus share with the animal protection test the disadvantage of possible biological irregularities. Precipitin tests, however, are carried out with a reproducible non-cellular antigen of a definite chemical composition.

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Method.

The precipitin indices for a number of antipneumococcus sera and antibody preparations were determined by the precipitin tests described in the preceding paper. The study of the zonal phenomena permits the elimination of these irregularities.

Only the higher values furnished by multiplication of serum and precipitinogen dilution were used in calculating the precipitin indices, all individual values smaller than one-half of the maximal value were disregarded in estimating the average of each test. Precipitin index is a millionth part of the product obtained by multiplying the dilution of the antiserum by the dilution of the antigen. For details see the preceding paper.

TABLE I.

Comparison of Precipitin and Protective Antibodies in Monovalent Type I Sera and Antibody Preparations.

No. of serum	Precipitin index	Protective units	Ratio	Reference
155	400	100	4.0	Table IX (*d*)
47	560	200	2.8	Table IX (*d*)
169	1250	200–400	4.1	Table IV
90	700	200	3.5	Table VII preceding paper
32	1720	400	4.3	Tables I and VI preceding paper
32	2550	800	3.2	Tables III and XI preceding paper
	2880†		3.6	
94	3840	800	4.8	Table IV
Preparation "f"	2340	800	2.9	Table IV
102	7170†	800	9.0	Table X preceding paper
916	1920	200	9.6	See page 88
Average ratio for the first nine values.....			3.8	

† With addition of 10 per cent normal horse serum.

The proportions of the corrected precipitin index to the protective units of the same antibody solution were compared and an unanticipated constant relation was revealed.

The quotient precipitin index/protective units for a number of monovalent Type I horses ranged between 2.8 and 4.8 with an average of 3.7. For some monovalent Type III horses the

TABLE II.

Precipitin and Protective Antibodies in Monovalent Type III Sera.

No. of serum	Precipitin index	Protective units	Ratio	Reference
125	160	4, 8, 10	16–48	Table V preceding paper
	192			Table V
126	220	4	34–55	Table V preceding paper
	135			Table V
127	128	4	32	Table V
128	85	4–10	8–21	Table V
302	575	10	58	Table X
"A"	770	10	77	Table X
"B"	6150	80	77	Table X
Average ratio of all values except the two last.....			34	

TABLE III.

Relation between Precipitin Index and Protective Potency for Polyvalent Antisera.

Horse No.	Duration of treatment	Type I			Type III		
		P. I.	P. U.	Ratio	P. I.	P. U.	Ratio
	mos.						
3079	28	720	1000	0.72	>340		
3180	28	530	500	1.06	320	10	32
			1000	0.53		(20)	(16)
3332	22	530	400	1.35	>340	10	34
			500	1.06		(20)	(17)
3335	22	350	<500	>0.70	>190	(8)	(24)
3514	11	400	100	2.0	72	2	36
			200	4.0		(4)	(18)
3530	10	480	200	2.4	256	10	26
						(20)	(13)
3531	10	60	50	1.2	>192		
3635	6	240	50	2.4	45	2	23
			100	4.8			
3640	6	60	50	1.2	30		
3639	6	<40	50	<0.8	50	2	25
Average ratio	upper 4 horses.....			0.9			24
	lower 6 horses.....			2.3			

The parenthesized figures for protective units in Type III are based on 72 hours delay of death rather than on survival over 96 hours; on account of the pro-zone familiar in Type III pneumococcus mouse tests no survivals occurred.

quotient ranged between the extremes of 8 and 58 with an average of 34 (Tables I and II). When comparing these figures with those of Type I the almost ten times greater precipitin power associated with the protective potency in Type III is striking. If we discount this difference by referring the higher precipitin index per protective unit in Type III to the higher specific activity of equal weights of the precipitinogen rather than of the antibody, there is seen to be a similar ratio between precipitin and protective activity of the antisera for the two types investigated.

For a number of polyvalent horses[1] decidedly lower values for the quotient precipitin index/protective units were observed. In Type I they ranged between 0.7 and 2.4 with an average of 1.75 and with extreme values of 0.53 and 4.8. For Type III in polyvalent horses the quotient ranged among the lower values of those given above for monovalent sera of this type, the average being 24 (Table III).

A decrease in precipitin activity was also observed when adding one heterologous monovalent serum to an equal amount of another.

Serum 90, Type I shows a precipitin index of 700 and upon the addition of Serum 129, Type II, the precipitin index was only 60. Serum 126, Type III, decreased upon addition of No. 129 from 135 to 32.

If the Type I values of the polyvalent horses were arranged according to the duration of the treatment they could be divided into two groups; one comprising four horses under pneumococcus treatment for 2 years had quotients ranging below 1.35 with an average of 0.9, while another group of horses under treatment for no longer than 1 year had values above 1.2 with an average of 2.3.

This observation indicates that the duration of the treatment influences the proportion of the two antibody activities. This became evident from an experiment on two Type I horses whose immunization we observed from the onset. It was found that the quotients for bleedings after 37 days were about twice as high as those after 114 days; the protective potency reached greater values at a slower rate than the precipitin.

When the precipitin test was applied to refined antibody prepara-

[1] Samples of these were supplied through the kindness of Dr. Stanley Beard of the Lederle Antitoxin Laboratories, Pearl River, New York.

tions the quotients obtained were higher than from the crude sera in our experiments. We observed values as high as 9 in Type I and above 70 in Type III. There is some doubt whether this difference arises during the refining process by a selective fractionation or is simply due to a lesser proportionalism between potency and concentration in concentrated solutions.

When making due allowance for the various influences noted and

TABLE IV.

Precipitin Test of Antipneumococcus Sera, Type I, No. 94 and No. 169 and of Antibody Preparation "f" Prepared by L. D. Felton's Procedure (4).

Serum No.	Dilution of serum	Dilution of soluble specific substance in millions									Precipitin index	
		4	8	12	16	24	32	64	96	128	"P. I."	
94	20				−	−	−	−	−	−	(<320)	
					(pro-zone)							
	40				−	−	−	−	−	−	(<640)	
	80				+	+	+	±	−	−	3840	
				(post-zone)								3840
	120	−	−	−	−	−	+	−			3840	
169	20				+	+	+	+	−	−	1280	
	40				+	−	−	−	−	−	640	
	80				−	−	−	−	−	−	<1280	1250
	120	+	+	+	−	−	−	−			1440	
	160	+	+	+	−	−	−				1920	
	240	−	−	−	−	−	−				(< 960)	
"f"	20				+		+	±	±	+	2560	
	40				+		+	+	−	−	2560	2340
	60				+		±	±	−	−	1920	
	120				−		−	−	−	−	(<1920)	

discussed in the present and in the preceding paper a fairly accurate idea of the amount of antibody contained in an antipneumococcus serum can be obtained. The determination of the precipitin index may replace the slow mouse test in the course of immunizing horses as well as during refining processes; for final controls animal tests will have to be retained.

EXPERIMENTAL PART.

In the following tables data are collected for several monovalent Type I and Type III sera. Some of them like No. 94, Type I, Table IV, and No. 125 and No. 127, Type III, Table V, show a pro-zone and

TABLE V.

Precipitin Test of Antisera 125 to 128, Type III.

Dilution of serum	Dilution of soluble specific substance III in millions															P. I.
	0.05	0.1	0.2	0.3	0.4	0.6	0.8	1.2	1.6	2.4	3.2	4.8	6.4	9.6	12.8	
No. 125																
10	+	+	+	+	+	+	+	+	+	−	−	−				(16)
20	+	+	+	+	+	+	+	+	+	±	−	−				(40)
40	−	+	+	+	+	+	+	+	+	+	+	+				192
80		±	−	+	−	−	−									(24)
120		−	−	−	−	−	−									−
No. 126																
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	112
20	+	+	+	+	+	+	+	+	+	+	+	+	+	±	−	160
40	±	+	+	+	+	+	+	+	±	±	−	−				(64)
80		−	−	−	+	±	−									(40)
120		+	−	−	±	−	−									(42)
No. 127																
10	+	+	+	+	+	+	+	+	+	−	−	−				(16)
20	+	+	+	+	+	+	+	+	+	−	−	−				(32)
40	+	+	+	+	+	+	+	+	+	+	+	−				128
80		+	+	+	−	+	−									(48)
120		−	−	−	−	−	−									−
No. 128																
10	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	64
20	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	96
40	±	−	+	+	+	+	+	+	+	+	−	−	−	−	−	96
80		+	±	−	−	±	−									(40)
120		±	+	+	±	+	−									72

in these instances only the high values obtained from higher serum dilutions were taken into consideration. Values lower than 50 per cent of the highest value in a test are parenthesized and were disregarded.

In Table VI experimental results for Type I and Type III of ten

polyvalent horses, immunized against three types, are given. The figures are the dilution products or precipitin indices for the individual serum dilutions given in the first column. Pro-zones of more or less extent, may be observed in Sera 3079, 3180, 3640 and others for both types, in Sera 3514 and 3530 for Type I, and almost in all sera for serum dilution 1:5 for Type III.

TABLE VI.

Synopsis of Precipitin Tests of Sera Obtained from Ten Polyvalent Horses (Types I, II, III).

Precipitin indices for single serum dilutions given. Figures in parenthesis disregarded in computation of average P.I.

Dilution of serum	Horse No.									
	3079	3180†	3332	3335†	3514	3530	3531	3635	3640	3639
					Type I					
5	(80)	(40)	–	320	(30)	(20)	40	(120)	(<20)	Less
10	(80)	(80)	–	320	(120)	(60)	60	(80)	(<20)	than
20	(80)	(160)	(240)	320	(160)	(160)	40	(40)	60	40
40	(160)	320	320	320	(160)	(160)	80	(80)	60	
80	480	640	640	320	320	320	(<40)	240	(<40)	
160	960	640	640	480	480	640	(<80)	240	(<80)	
					Type III				‡	‡
5	(64)	(>128)	–	>128	–	>128	>128	32	(8)	32
10	(64)	(96)	–	192	–	>256	>256	32	32	48
20	(48)	(128)	–	>256	64	256	192	64	32	64
40	256	256	256	256	64	384	(64)	(<24)	28	28
80	>256	192	>256	128	64	256	(128)	(<16)	(<16)	16
160	>512	>512	>512	(96)	96	(96)	(<32)	–	–	(<32)

† see Table VII. ‡ see Table VIII.

Examples for both regular and pro-zone sera of each type are given in Tables VII and VIII.

A synopsis of these values is given in Table III, page 81, where the precipitin indices as calculated from Table VI are listed in the third and sixth columns. The protective units in the fourth and seventh columns of Table III, and in the third columns of Tables I and II were obtained from mouse tests, carried out according to the government standards in the Research Laboratories, Department of Health, City of New

TABLE VII.

Reaction of Soluble Specific Substance Type I with Polyvalent Antipneumococcus Sera.

Serum 3335 (Very Regular Test).

Dilution of serum	Dilution of soluble specific substance in millions								
	0.5	1	2	4	8	16	32	64	128
5				+	+	+	+	+	−
10				+	+	+	+	−	−
20			+	+	+	+	−	−	
40			+	+	+	−	−	−	
80	+	+	+	+	−	−			
120	+	+	+	±	−	−			

Serum 3180 (Pro-Zone Type).

5				+	+	−	−	−	−
10				+	+	−	−	−	−
20			+	+	+	−	−	−	
40			+	+	+	−	−	−	
80		+	+	+	+	−	−		
120		+	+	+	−	−	−		

TABLE VIII.

Reaction of Soluble Specific Substance Type III with Polyvalent Antipneumococcus Sera.

Serum 3639 (Regular).

Dilution of serum	Dilution of soluble specific substance in millions							
	0.2	0.4	0.6	0.8	1.6	3.2	6.4	12.8
5				+	+	+	+	−
10				+	+	+	±	−
20			+	+	+	+	−	
40			+	±	−	−	−	
80	+	−	−	−	−			
120	−	−	−	−	−			

Serum 3640 (Pro-Zone).

5				+	+	−	−	−
10				+	+	+	−	−
20			+	+	+	−	−	
40			+	±	−	−	−	
80	−	−	−	−	−			
120	−	−	−	−	−			

TABLE IX.

Development of Precipitin and Protective Antibodies against Type I Diplococcus Pneumoniæ in Two Horses.

Dilution of serum	Precipitin indices for individual serum dilutions. Duration of immunizing treatment.							
	(a) 0		(b) 37		(c) 72		(d) 114 days	
	47	155	47	155	47	155	47	155
10			(60)	(120)	(<40)	(<40)	(30)	(160)
20			(80)	160	(80)	(40)	(40)	(60)
40	Nil	Nil	(120)	240	(160)	(80)	(240)	(80)
80			640	320	480	320	640	320
160			640	(<160)	480	640	480	480
Average P.I.			640	240	480	480	560	400
Protective units	Nil	Nil	80–100	40	80	100	200	100
Ratio	—		6.4–8.0	6.0	6.0	4.8	2.8	4.0

TABLE X.

Precipitin Test of Serum 302, Type III, and of Two Globulin Fractions A and B, Concentrated 16 Times and 14 Times Compared with the Original Serum 302.

Dilution of antibody solution	Dilution of precipitinogen in millions											P. I.
	0.2	0.4	0.8	1.2	1.6	3.2	4.8	6.4	9.6	12.8	25.6	51.2	
No. 302													
20	+	+	+	+	+	−	−	−	−	−	−		(32)
40	+	+	+	+	+	+	−	−	−	−	−		(128)
80	+	+	+	+	+	+	+	+	−	−	−		512
160	+	+	+	+	+	+	±	−	−	−	−		640
320	−	−	−	−	−	−	−	−	−	−	−		—
A													
80	+	+	+	+	+	+	+	−	−	−	−		(384)
160	+	+	+	+	+	+	−	−	−	−	−		(512)
320	+	+	+	+	+	+	−	−	−	−	−		1024
480	−	−	−	−	+	−	−	−	−	−	−		768
B													
160	+	+	+	+	+	+	+	+	+	+	+	±	6144
320	−	−	−	+	+	+	+	−	−	−	−	−	(1536)
480	+	+	−	−	+	+	−	−	−	−	−	−	(1536)
A+B													
160	+	+	+	+	+	+	+	+	+	+	+	±	Stronger reactions than B alone

York, by Miss G. M. Cooper under the direction of Dr. W. H. Park, and in the Testing Department of the Lederle Antitoxin Laboratories, Pearl River, New York, by Miss F. L. Clapp, to whom we have to express our thanks for their kind cooperation.

The influence of the duration of the treatment on the quotient precipitin index/protective power was studied in two monovalent horses, No. 47 and No.155, for which the experimental data are to be found in Table IX. The quotients were 6 to 8 after 5 weeks, 4.8 to 6.0 after 10 weeks and 2.8 to 4.0 after 16 weeks.[2]

Two globulin fractions were obtained by the ammonium sulfate method (6) from Serum 302 (Table X). The first was concentrated to one-sixteenth, the second to one-fourteenth of the original volume. The first fraction contained only ten mouse units per cc. like the starting material, in the second a concentration of 80 units was attained. The ratio precipitin/protection, already as high as 58 in the original serum, rose to 77 for the globulins. By mixing the two globulin fractions in equal amounts an effect was observed similar to those discussed on page 65 of the preceding paper.

The precipitin values for Preparation 916, Type I, 200 protective units (Table I), were

for a serum dilution of	10	1280
	20	2560
	40	160
	80	80
	160	160;

in this preparation, as in No. 102 of Table VI in the preceding paper, the precipitin activity was very high as compared with the protective potency.

SUMMARY.

The precipitin indices for a number of monovalent and polyvalent antipneumococcus sera were determined under known conditions, and found to vary as did the number of protective units.

The ratio precipitin index/protective units in monovalent sera was

[2] Forster (5), investigating the relation between the time elapsed since immunization and the precipitin titer, observed the development of a post-zone during the "secondary stage."

found to lie between 2.8 and 4.8 for Type I and to be about ten times greater for Type III.

Lower values were found in polyvalent horses and when mixing heterologous monovalent sera with each other.

The influence of the duration of treatment upon the quotient was studied.

Several refined and concentrated preparations showed a relative increase in precipitin activity.

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