QUANTITATIVE EXPERIMENTS WITH ANTIBODIES TO SPECIFIC PRECIPITATES. II*

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In the first article of this series (1) a quantitative study of the antigenic properties of antibodies derived from a variety of immune sera was described. These antibodies were used, largely in the form of specific precipitates, as test antigens against serum resulting from the injection of rabbits with a specific precipitate derived from antipneumococcus Type II horse serum. While the reactivity per milligram of the specific precipitates used as test antigens depended partly on their antibody:antigen ratios, the antigenic behavior of the various water-insoluble antibacterial globulins in antisera produced in the horse was identical in other respects. On the other hand, the water-soluble antibodies formed in the horse in response to injections of diphtheria toxin and crystalline egg albumin precipitated only 50 to 60 per cent of the antibody from the rabbit antisera to the Type II antipneumococcus specific precipitate.

Although it was not found possible to detect any specificity due to the anticarbohydrate groupings in the antibody injected into the rabbits (1), the present study was undertaken to investigate this aspect of the problem more closely. In order to ensure the production of antibodies against rabbit as well as horse specific precipitates these antigens were injected into chickens. The chicken antisera were tested with a number of the antigens previously used (1) in the study of the rabbit anti-precipitate sera.

EXPERIMENTAL

Preparation of the Specific Precipitate Suspensions for Immunization.—

1. S II[1]-Rabbit Anti-S II Specific Precipitate.—70 ml. of C[2]-absorbed Type II antipneumococcus rabbit serum containing 1.44 mg. of antibody N per ml. were diluted to

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[1] S, with the appropriate type numeral, used for the pneumococcus specific polysaccharide; Pn used for pneumococcus.

[2] C used for pneumococcus somatic carbohydrate.

225 ml. with 0.9 per cent saline and precipitated at 37°C. with 14 mg. of S II (2). After 15 minutes the precipitate was centrifuged at room temperature and washed five times at 37°C. with saline containing 1:10,000 merthiolate.[3] Ratio antibody N/S II in the precipitate[4] = 6.2.

2. S II-Horse Anti-S II Specific Precipitate.—This was the same as used previously (1). Ratio = 14.3.

Three chickens were injected intravenously with each suspension. 1 to 4 ml. of suspension, containing 1.5 mg. of protein per ml., were given in about 20 injections over

TABLE I

Precipitation of Antibody from Immune Chicken Sera by Specific Precipitates

Per 4.0 ml. serum, 0°C., 48 hours

Specific precipitate used as test antigen	Chicken antiserum to							
	Horse Pn II specific precipitate				Rabbit Pn II specific precipitate			
	Antibody N pptd. in successive absorptions			Total antibody N pptd.	Antibody N pptd. in successive absorptions			Total antibody N pptd.
	1	2	3		1	2	3	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Precipitates from horse sera:								
S II-anti-S II	0.164	0.027	0	0.19	0			0
S I-anti-S I	0.180	0.029	0	0.21	0			0
Diphtheria toxoid-antitoxin	0.096	0.011	0.002*	0.11*				
Ea-anti-Ea†	0.090	0		0.09				
Precipitates from rabbit sera:								
S II-anti-S II	0.006			0.01	0.280	0.169	0.002	0.45
S II-anti-S II‡					0.360	0.076	0.005	0.44
Ea-anti-Ea†	0.004			0	0.326	0.084	0	0.41
Pn C-anti-C					0.376	0.076	0	0.45

* 0.10 mg. antibody N per 4.0 ml. recovered from supernatant by addition of a horse Pn II specific precipitate suspension.

† Ea used for crystalline egg albumin.

‡ From aqueous solution.

a period of 6 weeks. The two sets of pooled sera were filtered through an L2 Chamberland filter, preserved with merthiolate (1:10,000), and neutralized to pH 7.0–7.1 before use.

Specific Precipitate Suspensions Used as Test Antigens.—The specific precipitates from horse sera were those used previously (1). The Pn C-anti-C specific precipitate was prepared by precipitation of a Type II antipneumococcus rabbit serum with C substance (3) of Type I pneumococcus. The S II-rabbit anti-S II specific precipitate was the same as that used for immunization. Although suitable for injection, the rabbit anticarbohydrate specific precipitate was difficult to grind to a sufficient fineness for accurate delivery from a pipette. For one of the experiments reported in Table I

[3] Manufactured by Eli Lilly and Company, Indianapolis.

[4] Assuming that all of the added antigen is in the precipitate.

(that marked aqueous solution) a more easily measured suspension was prepared by taking advantage of the solubility of rabbit specific precipitates in distilled water. After a preliminary washing of the stock specific precipitate several further washings with water were made. When only traces of salt remained the precipitates dissolved on stirring. Accurately measured amounts of the aqueous solution, of known antibody and polysaccharide content, were introduced into test tubes and an equal volume of 1.8 per cent saline was added, causing reprecipitation of the specific precipitate in finely divided form. The chicken serum to be examined was added and the determinations made as described in (1). In other instances the procedure consisted in adding a known amount of suspension N to an accurately measured volume of chicken serum at 0°C., allowing the mixture to stand in the ice box for 48 hours with occasional stirring, followed by centrifuging and washing with saline. The precipitate was analyzed for nitrogen by the micro Kjeldahl method and any amount greater than that introduced was taken as antibody N. Aliquot portions of the supernatant were again set up as before and absorption was continued until only the suspension N added was recovered.

DISCUSSION

It will be noted from Tables I and II that both S II-anti-S II horse specific precipitate and S I-anti-S I horse specific precipitate removed the same amount of antibody N from chicken antiserum to the former. This supports the conclusion reached previously (1) in the study of rabbit anti-horse specific precipitate serum that the antigenic reactivities of the water-insoluble antibodies from horse sera are the same and independent of their antibody specificities. The amounts of antibody N removed by the diphtheria antitoxin and horse anti-Ea specific precipitates were 57 and 47 per cent respectively of that removed by the S II-anti-S II specific precipitate. The extent of the cross reactions given by the specific precipitates derived from water-soluble antibodies in the horse is thus approximately the same for both the rabbit and the chicken antisera to the horse specific precipitate (Table II).

In the chicken antiserum to the S II-rabbit anti-S II specific precipitate the same amounts of antibody N were precipitated, within experimental error, by the anti-S II, Pn C, and Ea specific precipitates from rabbit serum. Rabbit antibodies to Pn type specific carbohydrates and to crystalline egg albumin have been shown to occur in the so called γ-fraction by electrophoretic analysis (4) and it is therefore of interest that they precipitate the same amount of antibody N from the chicken serum. Although the electrophoretic behavior of rabbit antibody to Pn C substance has not yet been examined the present study shows that this antibody belongs to the same immunological group.

In the previous paper (1) it was pointed out that the specific precipitate used for injection should be reactive with more S II, that is, it should contain free groupings with antibody function. If these were antigenic, the

anti-precipitate serum obtained might be expected to react with any specific precipitate having free groupings reactive with S II, even though the antibody molecules carrying the groupings were derived from a species other than that used in the immunization. Reactivity of this kind has not been observed, however, in the three instances tested: chicken antiserum to horse anti-S II specific precipitate tested with rabbit anti-S II specific precipitate, chicken antiserum to rabbit anti-S II specific precipitate tested with horse anti-S II specific precipitate, and rabbit antiserum to horse Pn II specific precipitate tested with rabbit anti-S II specific precipitate (Table II). The actual amounts of nitrogen apparently precipitated in

TABLE II

Antibody Removed by Various Specific Precipitates from Chicken and Rabbit Antisera to Specific Precipitates

Specific precipitate used as test antigen	Antibody N removed at 0°C., 48 hrs.		
	Chicken anti-Pn II (horse)	Chicken anti-Pn II (rabbit)	Rabbit anti-Pn II (horse)*
	per cent	*per cent*	*per cent*
S II-anti-S II (horse)	**100**	0	**100**
S II-anti-S II (rabbit)	3	**100**	0
S I-anti-S I (horse)	109	0	96
Pn C-anti-C (horse)			97
Pn C-anti-C (rabbit)		101	
Ea-anti-Ea (rabbit)	2	91	
Ea-anti-Ea (horse)	47		50
Diphtheria toxoid-antitoxin (horse)	57		60

* Data from Table IX, reference 1.

the first two systems are equal to zero within the experimental error involved in analyses by difference.

It is evident from a large literature (5) that certain proteins, of similar functional significance, may show quite strict species specificity (serum proteins, hemoglobin), a broad organ specificity (lens protein), or some combination of both (thyroglobulin (6), enzymes (7)). The available data show that antibodies do not exhibit any specificity attributable to their ability to combine with a particular antigen. The antibodies from horse sera have been shown by a number of investigations (*cf.* (1) for literature) to fall into two immunological groups which also differ in their solubility in water. In this paper evidence is presented that rabbit antibodies to three different substances remove the same amount of antibody N from a chicken antiserum to one of the antibodies and are therefore identical in this respect. Even though no antigenic specificity dependent on a par-

ticular antibody function could be demonstrated it does not follow that antibodies are immunologically or chemically identical with one or more globulins of normal serum of the same species. This point will be considered in a later paper.

SUMMARY

1. Antisera have been produced in chickens with specific precipitates from Type II pneumococcus horse and rabbit antisera.

2. Specific precipitates from anti-Types I and II pneumococcus horse sera removed the same amount of antibody from the chicken anti-horse specific precipitate serum. Specific precipitates from horse antisera to diphtheria toxin and to crystalline egg albumin removed about one-half of the antibody.

3. Specific precipitates from anti-egg albumin, antipneumococcus C substance, and anti-Type II pneumococcus rabbit sera removed the same amount of antibody from the chicken anti-rabbit specific precipitate serum.

4. No antibody was removed from the chicken anti-horse specific precipitate serum by rabbit specific precipitates or from the chicken anti-rabbit specific precipitate serum by horse specific precipitates.

5. It is concluded that the antigenic specificities of antibodies from the horse and rabbit are not influenced by their particular antibody functions.

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