ANTIPROTEINS IN HORSE SERA

IV. Antibodies to Rabbit Serum Globulin and Their Interaction with Antigen*‡

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In the preceding paper (2) it was shown that the subcutaneous injection of horses with rabbit serum albumin was followed by the production of antibody. This antibody resembled the diphtheria antitoxin and anti-egg albumin formed in horses in its solubility in water, its limited zone of flocculation with antigen, and its serological behavior toward an anti-antibody serum. It was also shown that precipitable antibody was not produced following intravenous injections of the same rabbit serum albumin.

Nevertheless, it had been found that intravenous injection of pneumococcal lung autolysates into horses elicited the formation of antibody to the pneumococcal protein (3), and that this antibody combined with antigen in a typical precipitin reaction devoid of the prezone characteristic of the reactions of the antitoxins and anti-albumins. It is now shown that intravenous injection of horses with another kind of protein antigen—in this instance rabbit serum globulin—results in the production of antibody giving a typical precipitin reaction. In addition, intracutaneous or subcutaneous injection of horses with rabbit serum globulins gives rise to antibody of the so called "univalent" (4), soluble, low grade, non-flocculating type.

EXPERIMENTAL

Injection of Horses.—The preparation of the alum-precipitated rabbit serum globulin and the mixed albumin-globulin suspensions used as antigens was described in reference 2. The four horses used were from the regular stock of the New York City Department of Health Research and Antitoxin Laboratory, Otisville, New York. The schedule of injections is given in Table I.

Electrophoretic Patterns.—Electrophoretic runs[1] were made with the sera of the horses injected with rabbit globulin. In the case of horse 1046 the areas of the γ-components were

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‡ A preliminary abstract of a portion of this paper appeared in reference 1.

[1] In the Electrophoretic Laboratory of the College of Physicians and Surgeons, under the direction of Dr. Dan H. Moore.

essentially the same before and after injection, 22.8 per cent and 24.8 per cent of the total, respectively. After intravenous injection with mixed albumin-globulin, the serum of horse 1126 gave a pattern in which the γ-peak was prominent. Neither postimmunization pattern showed the pronounced peak between the β- and γ-globulins which characterized the electrophoretic diagrams of the sera of horses 999 and 1127 after subcutaneous injection with albumin or albumin-globulin mixtures (2).

TABLE I

Injection Schedule of Horses Receiving Alum-Precipitated Rabbit Serum Globulin or Albumin-Globulin Suspensions

Horse No.	Details of injection					
	Antigen	Route	From	To	No. of injections	Average amount
						mg.
1046	Globulin	Intravenous	Dec. 31, 1939	July 5, 1940	64	45*
		Intracutaneous	Dec. 4, 1940	Mar. 7, 1941	32	25
		Subcutaneous	Mar. 10, 1941	July 2, 1941	38	200‡
	For later immunization see Table I, reference (2).					
999	Globulin	Subcutaneous	Oct. 28, 1941	June 9, 1942	51	240
1126§	Globulin + albumin‡	Intravenous	Oct. 28, 1941	June 12, 1942	48	100‖
1127§	Globulin + albumin	Subcutaneous	Oct. 28, 1941	June 12, 1942	48	100‖

* The first seventeen injections were of 100 mg. globulin each, the remainder 25 mg., with few exceptions.

‡ The dosage was gradually increased from 25 to 200 mg. during the first nine injections and then held at that level.

§ From Table I, reference 2.

‖ Dosage reduced for four injections because of febrile reactions. Dosage of globulin only; an equal amount of albumin was also present.

Examination of Sera for Antibody

Intravenous Series.—Three and one-half months after the start of the injections, serum from horse 1046 (bleeding Mar. 18, 1940) showed precipitating antibody. A concentrate of the total globulin was prepared from 1950 ml. of this serum by precipitation with ammonium sulfate and dialysis in the cold against 0.9 per cent NaCl. The final volume was 500 ml.

Antibody in this concentrate was determined by the quantitative precipitin method (5, 2), with γ-globulin electrophoretically separated from normal rabbit serum as test antigen. The results are recorded in Table II. Antibodies to rabbit serum components other than γ-globulin were undoubtedly present, since the total globulins, containing some albumin as well, were used as immunizing antigen. Tests with α- and β-globulins were made, but could not be interpreted since it was difficult to secure enough of these components to ensure at least their electrophoretic homogeneity. The rabbit γ-globulin, on the other hand, could be obtained in larger quantities. Its essential homogeneity in the Tiselius apparatus was demonstrated by a second mobility determination. Moreover, the results obtained could be

compared with those secured with antigens consisting of specific precipitates containing γ-globulin antibody.

Qualitative precipitin tests with the final bleedings of horse 1126 (Table I) indicated that these sera were similar in their behavior to the corresponding bleedings of horse 1046.

Influence of Temperature on the Reactivity of Antibody.—Two series of precipitin determinations were made on whole serum of horse 1046 at the conclusion of the intravenous schedule. One was set up at 0°C. and the tubes were allowed to stand 3 days in the ice box, with subsequent washing in the cold.[2] The other series was set up at 37°C. and the tubes were incubated for 2½ hours and centrifuged and washed at the same temperature. The data are given in Table III and plotted in Fig. 1.

Determination of Antibody by Absorption with Specific Precipitates.—Since anti-egg albumin in the rabbit is known to be a γ-globulin (6) and specific precipitates containing such antibody,

TABLE II

Precipitation of Horse Antibodies to Rabbit Globulin by Electrophoretic Rabbit γ Globulin

Per 8.0 ml. concentrate of serum 1046, bleeding Mar. 18, 1940, 0°C., 48 hours

Antigen N added	Antigen N precipitated	Total N precipitated	Antibody N precipitated (by difference)	Antibody N / Antigen N	Tests on supernatants
mg.	*mg.*	*mg.*	*mg.*		
0.019_2	Total*	0.144	0.125	6.5	Excess HA‡
0.038_4	"	0.242	0.204	5.3	" "
0.057_6	"	0.300	0.242	4.2	" "
0.077	"	0.350	0.273	3.5	No HA or γG
0.096	0.088§	0.382	0.294	3.3	Excess γG

Mg. antibody N precipitated = $9.3\ (\gamma G\ N) - 20.6\ (\gamma G\ N)^{3/2}$

Calculated to 1.0 mg. precipitable antibody N:

Mg. antibody N precipitated = $9.3\ (\gamma G\ N) - 10.9\ (\gamma G\ N)^{3/2}$

* All N of the gamma globulin solution was assumed to be antigenically active.

‡ HA = horse antibody; γG N = rabbit electrophoretic gamma globulin N.

§ From analyses on supernatant.

if properly washed, can be obtained free from other serum proteins (7), they provide a convenient source of γ-globulin, suitable for use as antigen (8, 9). The analytical procedure is similar to that of the quantitative agglutinin method (10): an accurately measured amount of a suspension of the washed specific precipitate, of known N content, is added to duplicate portions of serum. If the serum contains much antibody visible agglutination often occurs after the contents of the tubes are mixed. The tubes are centrifuged, washed 2 to 3 times with 0.9 per cent NaCl, and the precipitates analyzed for N. The excess of N found over that added is taken as antibody N. Absorption with fresh portions of precipitate is continued until no more antibody N is added. Since the antigen is virtually insoluble, inhibition reactions due to excess antigen are avoided.

Bleedings from horses injected by the various routes were analyzed by this method. A carefully washed, anti-egg albumin egg albumin specific precipitate with a high antibody to antigen ratio was used as antigen, with the results given in Table IV.

[2] In a refrigerated centrifuge supplied by the International Equipment Co., Boston, Massachusetts.

TABLE III

Effect of Temperature on the Precipitation of Horse Antibodies to Rabbit Globulin by Electrophoretic Rabbit γ Globulin

Per 5.0 ml. horse serum H 1046, bleeding July 25, 1940, 0° and 37°C.

Antigen N added	Total N precipitated	Antibody N precipitated (by difference)	Antibody N / Antigen N	Tests on supernatants
mg.	*mg.*	*mg.*		
	0° C., 3 days			
0.006_5*	0.074	0.067	10.3	Excess A
0.013*	0.123	0.110	8.5	" "
0.022‡	0.172	0.150	6.8	" "
0.033‡	0.190	0.157	4.8	" "
0.039	0.206	0.167	4.3	" "
0.043‡	0.217	0.174	4.0	Slight excess A, tr. γG?
Mg. antibody N precipitated = 14.4 (γG N) − 52.0 (γG N)$^{3/2}$. Calculated to 1.0 mg. antibody N: Mg. antibody N precipitated = 14.4 (γG N) − 21.0 (γG N)$^{3/2}$				
	37°C., 2½ hours			
0.013..	0.084	0.071	5.5	Excess A
0.022‡	0.120	0.098	4.5	" "
0.033‡	0.140	0.107	3.2	" "
0.043‡	0.158	0.115	2.7	" "
0.052‡	0.175	0.123	2.4	No A, tr. γG

Mg. antibody N precipitated = 8.6 (γG N) − 28.5 (γG N)$^{3/2}$

γG N = electrophoretic rabbit γ-globulin N.

* 10 ml. serum actually used for analysis.

‡ 6 ml. " " " " "

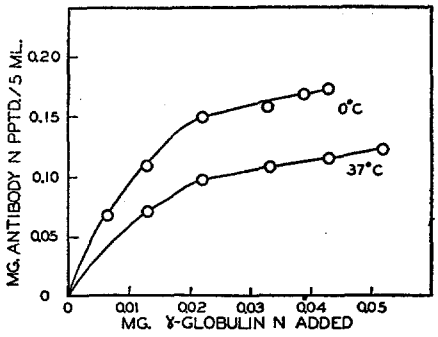

Fig. 1. Effect of temperature on the precipitation of horse antibodies to rabbit serum globulin by electrophoretically separated rabbit serum γ-globulin.

Sera Obtained after Intracutaneous and Subcutaneous Injections of Globulins.—Horse 1046 was rested for 5 months after the intravenous injections and then received a course of intra-

cutaneous injections of the same antigens (Table I). Although a bleeding taken Mar. 10, 1941, contained 0.11 mg. of antibody N per 5 ml. when analyzed with particulate γ-globulin antigen as above, it failed to precipitate with any of a number of dilutions of rabbit γ-globulin *solution*. This indicated that the antibody was univalent (4) or low grade.

Horse 1046 was next given subcutaneous injections of the rabbit serum globulins. Bleedings were taken July 2, 1941, and Oct. 28, 1941. The latter bleeding was negative, but the July 2, 1941, bleeding gave slight precipitation with relatively large amounts of γ-globulin (0.025 to 0.125 mg. N). Since the quantity of antigen used for maximum precipitation was

TABLE IV

Antibody Content of Anti-Rabbit Globulin Horse Sera as Determined by Quantitative Absorption with Anti-Egg Albumin (Rabbit) Specific Precipitates

Horse No.	Bleeding date	Injection*	Antibody N per 5 ml.
			mg.
1046	Feb. 26, 1940	Globulin, i.v.	0.17
"	Apr. 8, 1940	" "	0.18
"	July 25, 1940	" "	0.19‡
"	Oct. 21, 1940	End of rest period	0.11§
"	Dec. 23, 1940	Globulin, i.c.	0.10‖
"	Jan. 22, 1941	" "	0.07‖
"	Mar. 10, 1941	" "	0.11‡
"	July 2, 1941	" s.c.	0.33‡
"	Oct. 28, 1941	End of rest period	0.01‡
"	Feb. 4, 1942	Albumin, i.v.	0.09§
1126	Feb. 4, 1942	Albumin-globulin, i.v.	0.14§
1127	Oct. 28, 1941	Before injection	0.00
"	Feb. 4, 1942	Albumin-globulin, s.c.	0.38§
999	Oct. 28, 1941	Before injection	0.00
"	Feb. 4, 1942	Globulin, s.c.	0.41§

* i.v. = intravenously, i.c. = intracutaneously, s.c. = subcutaneously. Globulin, albumin, or albumin-globulin mixture used as indicated. Dosages given in Table I.

‡ Analyses on 4.0 ml. serum. All values recalculated to 5.0 ml. for comparison with other tables.

§ Analyses on 2.0 ml., recalculated to 5.

‖ Analyses on 3.0 ml., recalculated to 5.

about 1.5 times as much as the antibody content per milliliter determined with the specific precipitate (Table IV), it is probable that the observed reaction was due to a minor component such as β-globulin present as an impurity in the γ-globulin antigen.

The bleeding of July 2, 1941, was also tested for low grade antibody which could not be precipitated directly by soluble antigens but which could attach itself to specific precipitates containing fully active, multivalent antibody (*cf.* 4, 11 to 13). Accordingly, duplicate 5 ml. portions of precipitating serum of horse 1046 (bleeding July 25, 1940, after intravenous injections and similar to that in Table III) were set up at 0° in three series: one with added saline, another with 1.0 ml. portions of the bleeding of July 2, 1941, and the third with 4 ml. portions of this bleeding. To each set of mixtures appropriate amounts of antigen were added. The determinations were carried out in the usual manner and are recorded in Table V. The

amounts of additional precipitate N due to the coprecipitation of antibody in the July 2, 1941, bleeding are given in the last column of the table.

The specific precipitate method was used to analyze the sera from the remaining horses injected subcutaneously with rabbit serum globulin (No. 999) or with mixed albumin and globulin (No. 1127). As noted in Table IV appreciable amounts of antibody were present. The direct precipitation reactions with γ-globulin were difficult to interpret, however, since large amounts of antigen were required and the precipitates obtained were relatively small.

Fractionation of Antisera.—In order to study the distribution of the various antibodies between water-soluble and water-insoluble fractions of the globulins of the antisera, 34 ml. of the concentrate of the serum of horse 1046 (Mar. 18, 1940) used in the experiment recorded

TABLE V

Addition of Non-Precipitating Antibody from H 1046, Bleeding July 2, 1941, to Precipitates Formed from Rabbit γ-Globulin Antigen and Horse Serum H 1046, Bleeding July 25, 1940

Bleeding July 25, 1940	Bleeding July 2, 1941	Antigen N added	Total N precipitated	Differences from blank
ml.	*ml.*	*mg.*	*mg.*	*mg.*
		0°C. 3 days		
5.0	1.0	0.013	0.132	0.009
5.0	4.0	0.013	0.138	0.015
5.0	0	0.013	0.123	—
5.0	1.0	0.039	0.240	0.034
5.0	4.0	0.039	0.272	0.066
5.0	0	0.039	0.206	—
5.0	1.0	0.129	0.334	0.082
5.0	4.0	0.139	0.486	0.234
5.0	0	0.129	0.252	—
5.0	1.0	0.387	0.322	0.044
5.0	4.0	0.387	0.410	0.132
5.0	0	0.387	0.278	

in Table II were dialyzed against 3 daily changes of 400 ml. of 0.005 M phosphate buffer at pH 6.8. The precipitate (A) was centrifuged off and redissolved in saline. The solution gave an immediate precipitate with rabbit γ-globulin. The supernatant from precipitate (A), after addition of salt, was analyzed with a rabbit anti-egg albumin specific precipitate suspension and found to contain 44 per cent of the antibody originally present. By difference, 56 per cent of the antibody had been precipitated on dilution with water, a proportion somewhat lower than usual with the water-insoluble pneumococcus anticarbohydrate in the horse (14).

Another fractionation was carried out with a late bleeding (July 25, 1942) of horse 1127, which had received mixed albumin and globulin subcutaneously. The serum was precipitated with ammonium sulfate and the fractions coming down at ⅓ saturation, and between ⅓ and ½ saturation were each divided into water-insoluble and soluble fractions. The reaction of one of these (serum 1127 J, ⅓ to ½ saturated water-soluble) with rabbit serum albumin has been described in reference 2. The percentages of the total antibody recovered, as determined by analyses with an egg albumin anti-egg albumin specific precipitate, were: from the water-

insoluble portion of the fraction precipitated by ⅓ saturation with ammonium sulfate, 13 per cent; from the water-soluble portion, 23 per cent; from the water-insoluble portion of the fraction precipitating between ⅓ and ½ saturation, 2 per cent; from the water-soluble portion, 62 per cent. The water-soluble antibodies, which, in other experiments (2), reacted with soluble rabbit albumin as do antitoxins with toxins, comprised 85 per cent of the total.

DISCUSSION

The production of antibacterial (anticarbohydrate) antibodies by the intravenous injection of horses has been shown to be correlated in most instances with an increase in the amount of electrophoretic γ-globulin (15, 16), with occasional instances in which pneumococcus anticarbohydrate occurred in a new component (β_2 or T) with mobility between those of the β- and γ-globulins (6, 16, 17). On the other hand, antitoxin produced in the horse occurs almost exclusively in this new fraction, absent in most normal horse sera (18, 19).

The electrophoretic patterns obtained in the present series of studies are, in general, those to be expected from the earlier work quoted. No indication is found in the patterns obtained with the sera of either horse 1046 or 1126 of the formation of a new component with mobility between the β- and γ-components. These horses received intravenous injections. In contrast, the patterns for sera 999 and 1127 clearly showed the formation of a new β_2 or T component after subcutaneous injections of rabbit serum albumin. The antigens and the injection schedules for horses 1126 and 1127 were identical; only the routes of injection were varied (Table I), horse 1126 having been injected intravenously, horse 1127 subcutaneously with the same albumin-globulin mixture (*cf.* (2)).

The antibody present in the serum of horse 1046 after 4 months of intravenous injections with rabbit globulin gave a typical precipitin reaction with a soluble antigen—normal rabbit electrophoretic γ -globulin. This reaction (Fig. 1) is of the type given by pneumococcus anticarbohydrate (20) and antiprotein (3) in the horse. Characteristic is the absence of a prezone in the region of antibody excess; instead, the curves may be extrapolated to the origin. This is, of course, in marked contrast to the behavior of the rabbit serum albumin–horse anti-albumin system (2), and other examples of the so called flocculation reaction (11, 19, 21, and 22).

The data for the globulin anti-globulin system are best represented by an empirical equation (Table II) involving the first and the 3/2 power of the quantity of antigen added and precipitated, as first proposed for several other precipitating systems involving antiprotein formed in the rabbit (23, 4, 8, 9). Although this equation has not yet been derived from fundamental considerations, as has another which best represents numerous other systems (5*b*, 20*d*) it has the merit in these instances of fitting linearly a plot representing the ratio of antibody N: antigen N precipitated against the square root of the amount of antigen N added. For comparison of different sera, the data are recalculated to a common antibody content, for example, 1.0 mg. N per ml.

When the bleeding taken from horse 1046 after a 7.5 month intravenous

course was set up with rabbit γ -globulin at two temperatures, 0° and 37° (Table III), a rather marked variation of reactivity with temperature was noted. The antibody precipitable at 37° was only 0.123/0.174, or 71 per cent of that removed at 0.° This resembles closely the findings obtained with anticarbohydrate systems in the horse (20 *c*) and in the rabbit (24). On the other hand, precipitating antiprotein (anti-egg albumin) in the rabbit (4) or flocculating antiprotein systems in horse sera (rabbit serum albumin (2); egg albumin (11, 12); diphtheria toxin (21)) have practically negligible temperature coefficients.

The maximum combining ratio (20 *d*, 4) at 37°—obtained by extrapolation to zero antigen N of the line giving the variation of the ratio antibody N: antigen N precipitated with antigen N added—is also less than at 0°. At the higher temperature it is calculated that only 8.6 mg. of antibody N can be removed per mg. of γ-globulin N, compared with 14.4 mg. at 0°.

As shown in Table IV, the total amount of antibody to γ-globulin, determined at 0°, increased very little (0.18 to 0.20 mg. N/5 ml.) in test samples of the serum of horse 1046 during the last 3 months of the intravenous injections. The quantitative properties of the antibodies show appreciable differences, however. Since the data for Table II were obtained on a dilution of a globulin solution, while those for Table III were on whole serum of different antibody content, it is necessary to compare them on some common basis, such as 1.0 mg. of precipitable antibody N. When this is done the following equations are obtained:

	Bleeding date 1940	Mg. antibody N precipitated =
(1)	Mar. 18	9.3 (γG N) minus 10.9 (γG N)$^{3/2}$
(2)	July 8	16.3 (γG N) minus 26 (γG N)$^{3/2}$
(3)	July 25	14.4 (γG N) minus 21 (γG N)$^{3/2}$

It is evident from the above that the initial combining ratio (9.3) and the slope (10.9) characteristic of the March 18, 1940, bleeding (equation (1)) are both significantly lower than the corresponding constants for the later bleedings (equations (2 and 3)). The changes in these two factors are in accord with other quantitative data (4, 12) and with general experience that the reactivities of antibody frequently tend to broaden on progressive immunization.

It will be noted from Table IV that the antibody concentration in the serum of horse 1046 dropped from 0.18 to 0.10 mg. N per 5 ml. at the end of the rest period following intravenous injection of globulin. The antibody content remained practically constant after a series of intracutaneous injections (bleeding March 10, 1941) and then increased markedly after a further course of subcutaneous injections to 0.33 mg./5 ml. (July 2, 1941). This increase, however, was due to the gradual replacement of the precipitating antibody by "univalent" antibody (4, 11, 12) which did not precipitate with soluble antigen. The value given was obtained by addition to the serum of a washed specific precipitate composed of egg albumin and rabbit anti-egg albumin. This

antibody has been shown to be in the γ-globulin fraction (6) of rabbit sera. This device consequently permitted the use of rabbit γ-globulin in an insoluble form with which the "univalent" antibody could combine and be measured quantitatively.

That the precipitating or "multivalent" form of antibody should not recur during the intracutaneous and subcutaneous injections subsequent to the rest period was indeed unexpected, especially since new antibody of low grade or "univalent" reactivity was produced. Mixtures in various proportions of earlier precipitating bleedings with the non-precipitating antibodies actually gave precipitates with soluble γ-globulin (Table V), providing evidence against any markedly inhibitory action of the "univalent" antibody which might mask the presence of a small amount of residual precipitating antibody. Failure of the precipitating antibody to reappear when the route of injection was changed points strongly toward the essential independence of the physiological mechanisms for producing the two forms of antibody.

When the antibody is removed by attachment to preformed precipitates (egg albumin anti-egg albumin) (Table VI), lower values of the constants are obtained than in Table III, possibly because only the rabbit γ-globulin molecules at the surfaces of the particles are available for interaction with the anti-globulin in the horse serum, or perhaps because of masking of portions of the rabbit globulin configuration by the egg albumin.

While most of the antibodies formed in horses after the subcutaneous injection of rabbit serum albumin are to be found in the water-soluble fraction of the globulins of the antisera (2), the antibodies developed in response to the intravenous injection of rabbit globulin are largely water-insoluble. The quantitative reaction curves (Tables II and III) are also similar to those obtained with bacterial carbohydrate-anticarbohydrate systems in horse sera (20 *c*, *d*) and show the same marked temperature coefficient. The antibody formed in response to subcutaneous injection of globulin differs most strikingly from that produced after intravenous injection in its failure to precipitate with globulin in solution.

Since it has now been amply shown that the zonal type of flocculation is not the only type of reactivity possible in antiprotein systems in horse sera the older classification into anticarbohydrate and antiprotein reactions therefore appears to be an oversimplification. According to Kendall (25) the differences in reactivity between the precipitin type of antibody and the zonally flocculating antitoxin can be accounted for quantitatively by the assumption that in the former molecule two groups reactive with antigen (bivalent antibody) are alike, and that in the antitoxin molecule the two groups differ in affinity.

Another instance is also provided of the occurrence of low grade antibody free from the precipitating form with which it usually occurs. Horse anti-egg albumin with this property has previously been described (11, 12) as occurring

in an early stage of the immunization, while serum from later bleedings gave a characteristic zone of flocculation.

It is accordingly clear that the horse can produce a number of antibodies with differing chemical, physical, and serological properties. The route of injection and the nature of the antigen are major factors in determining the type of re-

TABLE VI

Absorption of Antibodies to Rabbit Globulin in Sera of Horse 1046 by Means of a Specific Precipitate

Per 5.0 ml. horse serum H 1046, 0°C., 3 days

Egg albumin rabbit anti-egg albumin suspension N added	Bleeding July 25, 1940, visibly reactive with a soluble antigen (as in Table III)			Bleeding July 2, 1941, non-reactive with a soluble antigen (see text, pp. 99, 100)		
	Total N precipitated	Antibody N (by difference)	$\frac{\text{Antibody N}}{\text{Suspension N}}$	Total N precipitated	Antibody N precipitated (by difference)	$\frac{\text{Antibody N}}{\text{Suspension N}}$
mg.	*mg.*	*mg.*		*mg.*	*mg.*	
0.106*	0.203	0.097	0.92	0.203	0.097	0.92
0.213*	0.370	0.157	0.74	0.377	0.164	0.77
0.283‡	0.455	0.172	0.61	0.475	0.192	0.68
0.425	0.606	0.181	0.43	0.692	0.267	0.63
0.638	0.834	0.196	0.31	0.918§	0.280	0.44

Reaction equations‖

Mg. antibody N precipitated = 1.43 (S N) − 1.54 $(S N)^{3/2}$. A maximum (calculated) = 0.18 mg. N	Mg. antibody N precipitated = 1.26 (S N) − 1.04 $(S N)^{3/2}$. A maximum (calculated) = 0.27 mg. N
Conversion to basis of 1.0 mg. maximum precipitable antibody N, A, gives Mg. antibody N precipitated = 1.43 (S N) − 0.65 $(S N)^{3/2}$	to 1.0 mg. A N: Mg. antibody N precipitated = 1.26 (S N) − 0.54 $(S N)^{3/2}$

A = antibody.

* Double quantities of antigen and serum actually used.

‡ One and one-half quantities actually used.

§ One determination only.

‖ S N = antigen suspension N added.

sponse. Rabbit serum albumin does not appear to be antigenic in the horse when administered intravenously but leads to the formation of the antitoxic type of antibody when given subcutaneously. Rabbit serum globulin, on the other hand, functions as an antigen by both routes, but stimulates the production of precipitating antibodies only when injected intravenously.

It is not clear what property of the antigen might be concerned in these effects. Egg albumin, serum albumin, and diphtheria toxin are of lower molecular weight than serum globulin, which produces precipitating antibodies in

the horse, but molecular size cannot be the sole decisive factor since the subcutaneous injection of horses with hemocyanin (molecular weight 7,000,000) results in antibody of the antitoxin type (26). Clarification of this problem must therefore await further study.

SUMMARY

1. The intravenous injection of two horses with alum-precipitated rabbit serum globulin resulted in the production of antibody which gave a typical precipitin reaction without a prezone in the region of antibody excess.

2. The chemical, physical, and serological properties of this antibody are comparable to those of the more familiar anticarbohydrate antibodies.

3. The subcutaneous injection of horses with the globulin antigen gave rise to low grade "univalent" antibody which did not precipitate with soluble antigen.

4. The low grade antibody could be removed from solution by attachment to preformed specific precipitates, or by coprecipitation in the presence of "multivalent" precipitating antibody.

5. It is concluded that the familiar antitoxin type of antibody is not the only form of antiprotein response in horses but that precipitating and low grade non-precipitating antibodies may also be formed.

6. The nature of the antigen and the route of injection are demonstrated to be important factors in determining the characteristics of the antibody formed.

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