QUANTITATIVE STUDIES ON THE PRECIPITIN REACTION

Effect of Salts on the Reaction*

By MICHAEL HEIDELBERGER, Ph.D., FORREST E. KENDALL, Ph.D., and TORSTEN TEORELL,** M.D.

(From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York)

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In the studies on the precipitin reaction hitherto published from this laboratory (1) the salt concentration was held constant at 0.9 per cent of sodium chloride in order to permit observations on the effect of varying the proportions of antigen (or hapten) and antibody. In the present paper are reported data obtained during several years on the influence on the course of the precipitin reaction of changes in the concentration of sodium chloride and on the effect of other cations and anions.

According to our quantitative theory of the precipitin reaction, expressions of the form,

$$\text{mg. antibody N precipitated} = 2RS - \frac{R^2S^2}{A}$$

may be used to describe the course of the precipitin reaction (2). In these equations S refers to the specific polysaccharide or antigen, R to the ratio between antibody nitrogen and S precipitated at a definite reference point in the "equivalence zone" (2), and A to the antibody nitrogen precipitated at the reference point. The effect of increased salt concentration on these equations is also discussed in the present paper.

EXPERIMENTAL

The analytical technique used was that described in previous papers of the series (1–3). In the experiments summarized in Table I the final concentration

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of salt was varied from 0.6 per cent to 10.45 per cent, or 0.1 M to 1.79 M. 1.0 ml. of antibody solution B 36, prepared according to Felton (4) and made up with 0.9 per cent saline, was mixed in each case with the given amount of the specific polysaccharide of Type III pneumococcus (S III) dissolved in 3 ml. of salt solution of such strength that the final concentration of salt was as indicated in the table. The experiments were run for 2 hours at 37° and overnight at 0°, so that the results do not represent the maximum amount of antibody precipitable by any of the quantities of S III used (3). However, the amount of antibody nitrogen precipitable by a given quantity of S III decreased with increasing salt concentration, as did also the total amount of antibody nitrogen precipitable.

In Table II are given results of experiments run separately at 0° and at 37° in 0.9 per cent and 10 per cent sodium chloride solutions, using S III and Type III antipneumococcus horse serum 607 which had previously been precipitated with C substance and pneumococcus protein. 1.0 ml. of antiserum was added to a mixture of 1.0 ml. of S III solution in water and 2.0 ml. of salt solution of such strength as to give the desired final concentration. The tubes run at 0° were allowed to stand for 48 hours, except those containing excess S III, for which 4 days were allowed, while those at 37° were centrifuged after 2 hours, experiments having shown no significant differences between the amounts of antibody precipitated at 37° in 0.5, 1, 2, 4, or 6 hours.

Table III summarizes the data obtained with a divalent cation and divalent and tetravalent anions in the S III-antibody system. In this series of experiments 3.0 ml. of stock salt solution, 1.5 ml. of S III solution in water, and 1.5 ml. of antibody solution B 65 were mixed.

Table IV deals with the effect of salts on the reaction between crystalline egg albumin (Ea) and rabbit anti-egg albumin serum.

DISCUSSION

The data summarized in Table I show that at five different sodium chloride concentrations ranging from 0.1 to 1.79 molar, the amount of antibody nitrogen precipitated by a given quantity of S III from homologous antibody solution decreases with increasing salt concentration. This progression is reflected in a decrease in both constants of the equation for antibody precipitated, derived according to Reference 2. A similar effect is shown in whole serum both at 0° and at 37° (Table II), and it will be noted that the differences between the amounts of antibody precipitated from 0.15 and 1.75 M salt solution are approximately the same at both temperatures.

In an attempt to find the reason for the decrease in the amount of nitrogen precipitated at higher salt concentration the supernatants from the precipitate formed by the interaction of 0.07 mg. of S III and

TABLE I

Effect of the Concentration of Sodium Chloride upon the Reaction between S III and Antibody. 37° and 0°

Final NaCl concentration.....	Horse antibody solution B 36					Rabbit antibody solution B 50*	
	0.1 M	0.15 M	0.51 M	0.93 M	1.79 M	0.15 M	0.93–0.98 M
S III used	Nitrogen precipitated						
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
0.02	0.54	0.50	0.42	0.39	0.36		
0.05	1.13	1.03	0.90	0.84	0.75	0.43	0.24
0.075	1.41	1.41	1.29	1.15	1.03	0.60	
0.10	1.75	1.66	1.54	1.28	1.22	0.77	0.34
0.15	1.78	1.86	1.62	1.50	1.45	1.04	0.39
0.20†	1.82	1.85	1.70	1.58	1.51	1.18	0.41
Equations:‡ mg. antibody N pptd.....	27.5 S–104 S^2	25 S–84 S^2	22.2 S–72 S^2	20.2 S–68 S^2	18.1 S–57 S^2	9.5 S–18 S^2	5.0 S–15 S^2
A§.....	1.82	1.86	1.71	1.50	1.44	1.25	0.42

* Prepared according to Felton (5).

† Excess S III.

‡ *Cf.* Reference 2.

§ Calculated mg. antibody N pptd. at antibody excess end of equivalence zone.

serum 607 in 1.75 M salt solution (Table II) were combined and dialyzed at 0° against 0.15 M salt solution. According to the equation in column 2, Table II, 0.07 mg. of S III should precipitate 1.08 mg. of antibody N in 0.15 M salt solution at 0°. Thus, if the effect in 1.75 M salt solution were one of solubility, the decrease in salt concentration

TABLE II

Effect of Varying Sodium Chloride Concentrations on Reaction between S III and Antiserum 607 at 0° and at 37°

S III used	Antibody N precipitated at 0°		Antibody N precipitated at 37°	
	0.15 M NaCl	1.75 M NaCl	0.15 M NaCl	1.75 M NaCl
mg.	*mg.*	*mg.*	*mg.*	*mg.*
0.03		0.48		0.42
0.04	0.74		0.64	
0.07		0.83*		0.78
0.075	1.12	0.91	0.97	
0.10	1.24	1.01	1.09	0.87
0.15†		1.07		0.91
0.30†		1.10		0.94‡
1.00†		1.08		0.85
2.34†		0.87		0.54
Equations:§				
mg. antibody N pptd.....	22.5 S–101 S^2	18.4 S–83 S^2	19.3 S–85 S^2	16.2 S–75 S^2
A‖.....	1.25	1.02	1.10	0.87

* The supernatant remained clear when dialyzed at 0° against 0.9 per cent saline. The nitrogen determinations recorded in Tables II, III, and IV were run according to Teorell (6).

† Excess S.

‡ One determination.

§ *Cf.* Reference 2.

‖ Calculated mg. antibody N pptd. at antibody excess end of equivalence zone.

due to the dialysis should have caused 1.08–0.83, or 0.25 mg. of antibody nitrogen to precipitate for each of the supernatants which were combined and dialyzed. However, the solution remained clear, although it still contained antibody N which precipitated when more S III was added after the dialysis. It is therefore probable that the decreased amount of antibody precipitated at the higher salt concen-

trations is not due to increased solubility of the specific precipitate, but rather to a shift in the equilibrium brought about by the presence of the salt, by which the same amount of S III combines with a smaller quantity of antibody. This point will be discussed below in greater detail.

The data summarized in Table III show no very clear effect of a divalent cation or anion on the final result other than that of the total ion concentration, unless the approximately equal influence of 0.51

TABLE III

Effect of Varying Cations and Anions on Reaction between S III and Antibody Solution B 65

S III used	Antibody N precipitated				
	0.15 M NaCl	0.51 M NaCl	0.31 M $MgCl_2$	0.46 M Na_2SO_4	0.35 M $K_4Fe(CN)_6$
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
			Experiments at 0°		
0.020	0.43	0.39	0.34	0.35	0.40
0.045	0.76	0.58	0.57	0.61	0.68
0.150*	0.71	0.65	0.65	0.66	0.76
			Experiments at 37°		
0.020	0.40		0.27		
0.045	0.68	0.45	0.47†	0.55†	0.62
0.150*	0.72	0.59	0.62		0.62†

0.51 M NaCl, 0.31 M $MgCl_2$, 0.46 M Na_2SO_4, and 0.35 M $K_4Fe(CN)_6$ were calculated to give approximately the same osmotic pressure, equivalent to 1.8° depression of the freezing point of water.

* Excess S.

† One determination.

molar sodium chloride and 0.31 molar magnesium chloride ($MgCl_2$) be ascribed to the cation effect alone. The tetravalent ferrocyanide ion definitely counteracted the salt concentration effect. In addition it was observed that the magnesium ion decreased the flocculation velocity of the specific precipitate while the anions of higher valence increased the velocity, even in the zone of partial inhibition.

From Table IV it will be seen that the egg albumin-antibody reaction was found relatively insensitive to variations in salt concentration, contrary to the findings of Downs and Gottlieb (7) in qualitative

experiments with molar solutions, including sodium chloride, and with ferrocyanide.

In general, it may be said that the present observations on the effect of salts on the precipitin reaction are probably compatible with any of the theories which have been proposed for this reaction and are certainly not incompatible with our quantitative theory (1, 2, 8). It is not possible, however, to give a quantitative interpretation of the salt effects with the data at hand or with the methods which proved adequate at physiological salt concentrations. For example, it is possible

TABLE IV

Effect of Varying Salt Concentrations on the Precipitin Reaction between Egg Albumin (Ea) and Homologous Antibody, Serum 387 III, 1:1, at 0°*

Ea N used	Total mg. N precipitated					
	Cation effect				Anion effect	
	0.15 M NaCl	0.51 M NaCl	1.75 M NaCl	0.37 M $MgCl_2$	0.46 M Na_2SO_4	0.35 M $K_4Fe(CN)_6$
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
0.030	0.67	0.61		(0.53)	0.63	0.63
0.079	1.32	1.32	1.32	1.28	1.32	1.34
0.296†	1.03	−‡		−‡	0.95	1.09

* *Cf.* Reference 1.

† Excess Ea.

‡ The milky suspension deposited very little solid when centrifuged under the usual conditions.

that the opalescent solutions obtained in certain instances in the inhibition zone would have yielded more precipitate if centrifuged at higher speed. The electrolyte content of the precipitates is also unknown.

While the observed salt effects show certain similarities to those noted with typical globulins (9), the dialysis experiment quoted above (Table II) indicates that the diminution in precipitable nitrogen is not due to increased solubility of the specific precipitate. The cause appears to be rather a shift in the proportions in which S III and antibody combine. Whether this is due to a competition of the cation with antibody for combination with the S III anion (*cf.* 10), or a driving

back of the ionization of the soluble antibody-sodium chloride complex (*cf.* 11) is difficult to say. It is also possible that the large Coulomb forces due to the heaping up of carboxyl groups in the S III molecule (*cf.* 12) result in a spatial configuration which is altered when these charges are reduced by high salt concentrations, with a consequent diminution in specificity, or reactivity with corresponding spatially orientated groupings in the antibody molecule. At any rate there is much reason to ascribe an ionic mechanism to this effect, as was originally done in the case of the S III-antibody reaction itself (10). Salt effects similar to the above have also been noted by Hammarsten and collaborators (13), in the reaction between proteins and nucleic acids.

If the effect of high concentrations of salts on the precipitin reaction consists of a shift in the S III-antibody equilibrium, and if this shift is reversible, a method is available by which, with strong salt solutions, it should theoretically be possible to dissociate pure antibody from a specific precipitate formed at 0.9 per cent salt concentration in a suitable region of the reaction range. This phase of the work will be discussed in a paper now in preparation.

SUMMARY

1. A quantitative study has been made of the effect on the precipitin reaction between the specific polysaccharide of Type III pneumococcus and the homologous antibody of salt concentrations ranging from 0.1 M to 1.79 M, including the effect of ions of higher valence.

2. Within these limits, observed decreases in precipitated antibody with increasing salt concentration appear to be due to a decrease in the amount of antibody combined with the S III, rather than to an increase in solubility of the S III-antibody compounds.

3. The egg albumin-antibody reaction is far less sensitive to changes in salt concentration than is the S III-antibody reaction.

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