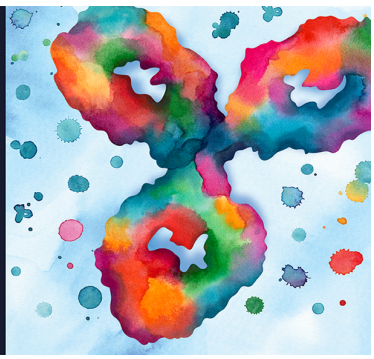

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Precipitin Production in Chickens: VI. The Effect of Varying Concentrations of NaCl on Precipitate Formation

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PRECIPITIN PRODUCTION IN CHICKENS

VI. The Effect of Varying Concentrations of NaCl on Precipitate Formation

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Chickens have been shown by earlier workers and by Wolfe, *et al.* (1, 2) to be excellent antibody producers. A much more consistent response to minute injections of antigen has been obtained from chickens than from rabbits. Use of these antisera has revealed that in some respects they cannot be handled the same as antisera produced in rabbits. Hektoen (3) found that a diluent of 1.8% NaCl for titrating fowl antisera by the interfacial method yielded much better results than the ordinary 0.9% diluent. Wolfe (1) and Wolfe and Dilks (4) found that with chicken antisera a marked *in vitro* rise of interfacial titer occurred and was completed within 12 days of the bleeding date. These results can be correlated with those of Moody, *et al.* (5) who found that freshly obtained chicken antisera also showed an *in vitro* rise of photroner titer (increased turbidity) when reacted on successive days. Similar effects have not been demonstrated in antisera produced in mammals. Further differences in the physico-chemical properties of fowl and mammalian antisera were presented by Deutsch, *et al.* (6). His work indicates that alpha globulin contributes to the antibody precipitating power of the gamma globulin of chicken antisera. This is not generally considered to be the case with mammalian antisera.

It is apparent that for the most effective handling of chicken antiserum a thorough study of the effect of physical factors is necessary. We are attempting such a study. The first factor being investigated is the effect of varying concentrations of NaCl on the reaction. The methods of study have been nitrogen and turbidity determinations of specific precipitates. Heidelberger, *et al.* (7) have demonstrated the value of the quantitative antibody method in determining the effect of varying concentrations of salts on the specific reaction of rabbit and horse antisera. Turbidity measurements, which can be performed rapidly and accurately by the photronreflectometer, have also been used effectively in similar studies by Boyden, *et al.* (8). In their study of various physical factors affecting the reaction of rabbit antisera, they demonstrated a close correlation between the turbidity and the nitrogen content of antigen-antibody precipitates.

MATERIALS AND METHODS

Crystalline beef albumin (Armour and Co.), human concentrated gamma globulin (Cutter Laboratories), and beef serum were the antigens used. 23 anti-beef-albumin sera were obtained from 115 chickens; some antisera were pooled samples and others were from individual birds. Three anti-human-globulin sera (one being a pool from two animals) and one anti-beef-serum (a pool from 12 animals) were also used. The injection procedure varied, but in most cases multiple injections of antigen were given.

Quantitative antibody determinations were made with some antisera. The method was essentially that of Heidelberger, *et al.* (9) with the exception that the salt concentration was varied and one washing of precipitate with 5.0 ml of saline was made. The N content was determined by the colorimetric method of Johnson (10). Turbidity measurements were made by the Libby photronreflectometer (11), according to the method described by Baier (12). In both methods serial dilutions of antigen were reacted with constant amounts of antiserum with saline added so that at each dilution the reaction occurred in a 2 ml mixture. The final salt concentration of the antigen-antibody mixtures varied from approximately 0.5 per cent to approximately 15.0 per cent. The antigen-antiserum mixtures were incubated for one hour at 37.5 ± 1 C. and were then kept overnight in the refrigerator. With some antisera these mixtures were kept for two days in the refrigerator, but there was no apparent difference in the results.

The supernatants, obtained by centrifuging the precipitates in the refrigerated centrifuge for one-half hour, were saved and tested for the presence of antigen and antibody. The usual test was by flocculation, in which an antiserum was added to a portion of the supernatant to test for antigen and antigen (from 2–4 μg N) to the remaining portion to test for antibody. A more precise determination of unprecipitated antigen was made by another method; in this method 0.5 ml of each supernatant was reacted with a constant volume of strong antiserum so that the reaction was in the region of antibody excess. Each mixture was made up to 2.0 ml with saline, and turbidimetric measurements were made with the photroner after incubation for one hour. This gave a relative measure of supernatant antigen, since the amount of turbidity was dependent upon the amount of antigen (see Table II). In order to secure the approximate nitrogen in the supernatant tests, antisera were calibrated for precipitation with known amounts of antigen. Turbidity measurements were then converted to gamma of antigen-N by interpolation. This method was based on the work of Libby (13) and Bukantz and Bullova (14), who showed the photroner could be successively used for determination of unknown amounts of antigen.

RESULTS

Typical results demonstrating the effects of various concentrations of sodium chloride on the chicken precipitin reaction are shown in Figure 1. With each increase in saline concentration a marked increase in the turbidity and in the precipitate nitrogen resulted. The region of greater precipitation occurred at the higher antigen concentrations. Similar results have been secured with all antisera studied.

A close parallel between the quantitative nitrogen and turbidimetric methods of assay is evident from Figure 1. The titration of other antisera by both these methods also showed this parallel. It thus seemed to us that the photroner assay could yield significant data, indicative of the N content of antibody-antigen precipitation. Since this method is simple and rapid it was used extensively in the present study.

A number of experiments were then performed in an attempt to find a basis for the high salt concentration effects. Our premise was that the increased precipitation in high salt concentration was due either to some non-specific reaction or to more complete antibody-antigen precipitation.

To determine the specificity of the precipitation with varying salt concentrations two types of controls were used. In one, antiserum and saline alone were reacted; in the other antigen was reacted with either normal chicken serum or completely heterologous antiserum. Table I-A records the results for 5 antisera

tested against saline alone. There were very small amounts of non-specific nitrogen with all the salt concentrations, but the amount in the 8% solution was never greater than in the 1%. When salt concentrations between 14% and 18% were used salting out of protein often occurred; at 18% it occurred in the 9 antisera tested. Two antisera were fractionated (to be explained later), and in the antibody containing fraction salting out was evident at 17% but not at 8 or 1%.

Table I-B records the reaction of an anti-human-globulin antiserum with beef albumin in 1.0 and 11.5% salt solutions. Only insignificant turbidity readings were obtained with the photroner. Similar results were secured when normal chicken serum was substituted for the anti-human-globulin serum. From the

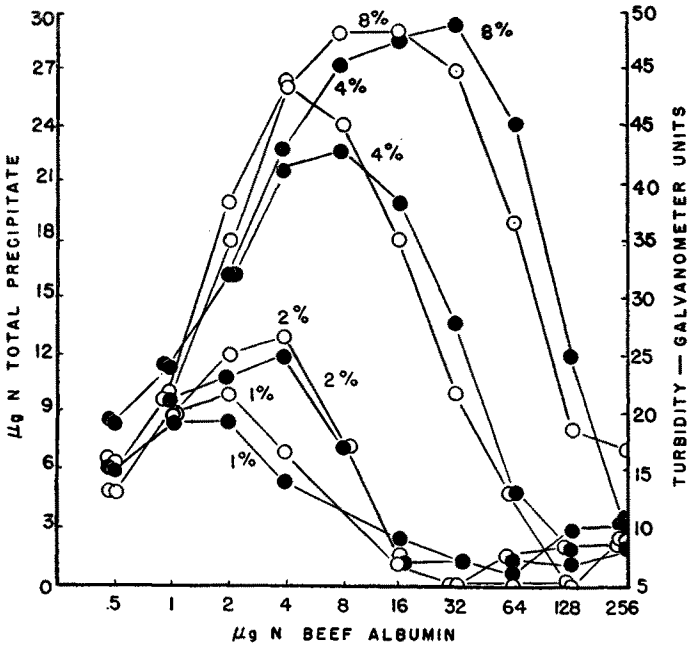

FIGURE 1. Comparison of techniques and salt concentration effects; —○—○— quantitative N data; —●—●— turbidimetric data. A pooled antibeef albumin (PC-129) × beef albumin — overnight readings.

above it is apparent that chicken sera cannot cause flocculation of antigen unless specific antibodies are present in the sera.

It has been suggested to us that the increased precipitation obtained in high salt concentrations was due to a co-precipitation of unrelated proteins with the specific precipitate. To test this possibility two chicken antisera were fractionated. The globulins were separated from the albumin by $\frac{1}{2}$ saturation with ammonium sulfate. The precipitate was dissolved and fractionated into water insoluble (euglobulin) and water soluble (pseudoglobulin) globulins by dialysis against distilled water. Each of the three fractions, after removal of the sulfate ions, was brought back to the original volume and tested for antibody. The albumin and

pseudoglobulin did not contain any detectable antibodies. The euglobulin of one antiserum showed an antibody content equivalent to the whole serum; the similar fraction from the other antiserum gave a decreased precipitation. The euglobulin fractions of both antisera gave much greater precipitation in 8% salt solution than in a 1%. Figure 2 and Table I show these results. It is thus evident that increased precipitation in the high salt concentration is not due to co-precipitation of the non-antibody proteins (albumin and pseudoglobulin). It may be added

TABLE I

Determination of the specificity of precipitates of chicken precipitin reaction

A. Antiserum plus varying concentrations of salt-quantitative technique

NaCl conc. of reaction	.25 ml antiserum + 1.75 saline				
	Euglobulin fraction of antibeef-albumin PC-125	Antibeef-albumin PC-134	Antibeef-albumin PC-129	Antihuman-globulin 11481	Antihuman-globulin 14329
	μg N				
%					
1	1 (5)*	7 (74)	7 (10)		
2			7 (13)	3 (35)	4.5 (43)
4		6 (108)	6 (26)		
8	1 (34)	4 (116)	5 (29)	4 (140)	4.5 (146)

B. Antiserum + heterologous antigen-turbidimetric method

NaCl conc. of reaction	Incubation period	.25 ml antihuman globulin no. PC 137 + 1.75 ml of beef albumin										
		μg N beef albumin										
		0	.5	1	2	4	8	16	32	64	128	256
%	*Hr.*											
1	1	0	1	2	1	1	0	1	1	0	2	1
	18	0	0	1	1	1	0	2	0	1	1	1
11.5	1	0	1	1	1	0	0	0	0	0	0	0
	18	0	0	1	0	0	0	0	0	0	0	0

* The number in parenthesis is the maximum N precipitated at the particular salt concentration when antiserum and homologous antigen were reacted; the control value of N was subtracted from the total N determined.

that Wolfe (unpublished) using the interfacial test also found that precipitins were present only in the euglobulin fraction.

The antigen-antibody precipitation curves have a definite and consistent pattern which further indicates that no co-precipitation occurs. They show that with increased salt concentration a significantly greater precipitation occurs only in the antigen-excess region and not in the antibody excess zone. Such data can be seen in Figures 1 and 2 and Table II. Table II shows that at antigen concentrations of 8 μg N and lower (antibody excess zone) the precipitation as determined by the photroner was similar in sodium chloride solutions of 1 to 10 per cent.

However, with higher antigen concentrations precipitation increased and supernatant antigen decreased on raising the salt content. This latter result is shown by the turbidimetric readings which represent relative amounts of unprecipitated antigen. Similar results were also obtained with a number of other antisera. Such data show that where increased precipitation occurred more antigen was precipitated.

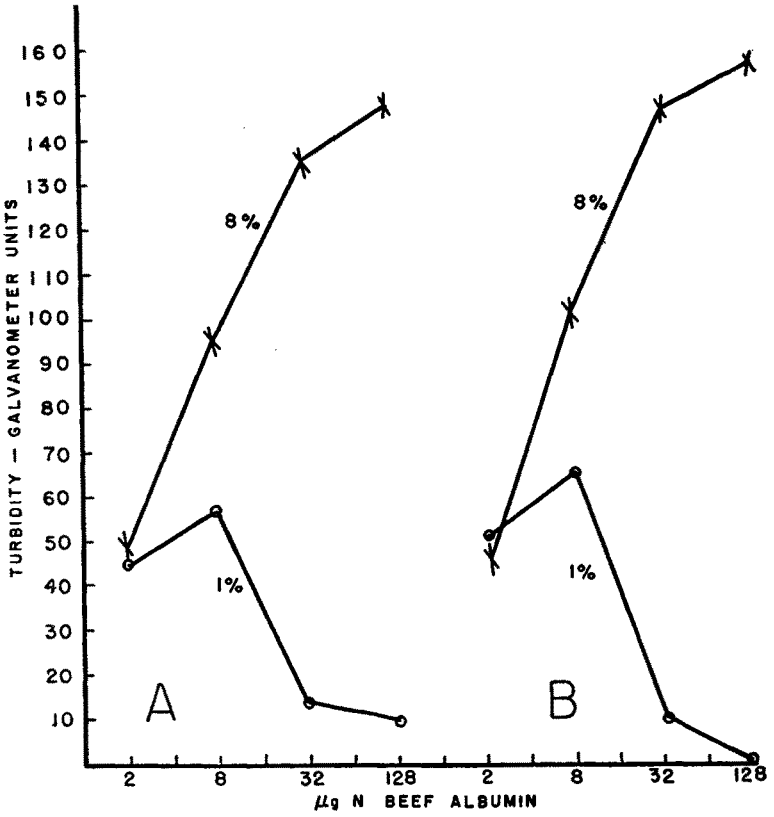

FIGURE 2. Effects of 1) varying the salt concentrations and 2) fractionation of antiserum on turbidity—in an antibeef-albumin-beef albumin system.

Curve A: .5 ml unfractionated antiserum PC-123 + 1.5 ml antigen — overnight readings.

Curve B: .5 ml euglobulin fraction of antiserum PC-123 + 1.5 ml antigen — overnight readings.

Another type of analysis corroborated the fact that increased precipitation in higher salt concentrations was a specific reaction. In this study the antigen to antibody ratios were compared at the points of maximal precipitation, which were always in antigen excess. Table III records the data for the reactions of an anti-human-globulin serum and an anti-beef-albumin serum with their homologous antigens in 1, 4 and 8 per cent salt solutions. The quantitative N technique

was used to determine the total amount of precipitate. The supernatant antigen N (column 7) was determined by the turbidimetric technique using a calibrated antiserum. The antibody N precipitated (column 5) was calculated by subtracting the precipitated antigen N (column 3) from the total precipitate N (column 4). The ratio of antigen N to antibody N (column 6) could then be easily determined.

TABLE II

Effect of salt concentration on turbidimetric measurements of an antibeef-albumin chicken serum (PC-108)—beef albumin system, including supernatant tests for antigen and antibody

SALT CONC.	KIND OF TEST*	TURBIDIMETRIC MEASUREMENTS—GALVANOMETER UNITS										
		μg N antigen per 2.0 ml of antigen-antiserum mixture										
		Cont.	0.5	1	2	4	8	16	32	64	128	384
%												
10	O	0	14	8	20	34	60	83	98	110	115	116
	A (10)				0	2	2	4	12	44	80	
	B (10)				+	+	+	−	−	−	−	
8	O	0	0	8	15	28	48	78	93	101	114	86
	A (8)				6	4	2	2	12	39	72	
	B (8)				+	+	+	−	−	−	−	
6	O	0	6	10	16	30	51	77	92	100	101	8
	A (6)				1	1	0	2	16	45	85	
	B (6)				+	+	+	−	−	−	−	
4	O	0	9	9	15	30	56	84	94	85	31	1
	A (4)				2	0	2	9	31	73	104	
	(2)				0	0	0	1	28	66	85	
	B (4)				+	+	+	−	−	−	−	
2	O	0	5	10	18	32	54	68	37	14	2	
	A (2)				2	4	8	23	50	80	92	
1	O	0	6	11	24	38	50	41	21	12		
5	O	0	2	9	16	26	28	18	7	1		

* O = overnight turbidity of original test; A = 1 hour turbidity in supernatant due to excess antigen; B = presence or absence of antibody; number in parenthesis is final salt concentration (%) used in testing supernatant.

The anti-human-globulin serum gave ratios at the points of maximum antibody precipitation (in slight antigen excess) of 4.7, 4.7 and 5.0 respectively with the 1, 4 and 8 per cent salt solutions. On the other hand, the total amounts of antibody N and of antigen N precipitated were greater in the higher salt concentrations than in 1.0%. Similar data were secured with the anti-beef-albumin serum also recorded in Table III. The fact that the ratios of the maximal precipitates were never greater in 4 and 8% NaCl than in 1% NaCl demonstrate the specificity of

TABLE III

Effect of salt concentration on the N ratio of antibody (Ab) to antigen (Ag) in the precipitate

SALT CONC.	ANTIGEN N ADDED	ANTIGEN N PPTD.	TOTAL N PPTD.	ANTIBODY N BY DIFFERENCE	RATIO ANTIBODY : ANTIGEN IN PPT.	TESTS ON SUPERNANATANT
.15 ml. antihuman globulin serum (No. 2940–2910) + 1.85 ml antigen solution						
%	μg.	μg.	μg.	μg.		
1.0	2.3	2.3	30.0	27.7	12.0	No Ab or Ag
	4.6	3.1	39.2	36.1	11.6	No Ab; slight Ag excess (1.5 μg. N)
	9.3	7.8	44.0	36.2	4.7	No Ab; slight Ag excess (1.5 μg N)
	18.5		32.5			Excess Ag (inhibition)
	37.0		24.5			Excess Ag (inhibition)
4.0	4.6	4.6	48.3	43.7	9.5	Excess Ab
	9.3	9.3	63.5	54.2	5.8	No Ab or Ag
	18.5	12.5	70.8	58.3	4.7	No Ab; slight Ag excess (6 μg N)
	37.0	13.0	69.3	55.3	4.2	Excess Ag (24 μg N)
	74.0		64.8			Excess Ag (inhibition)
8.0	4.6	4.6	48.3	44.0	9.5	Excess Ab
	9.3	9.3	60.8	51.5	5.5	No Ab or Ag
	18.5	12.5	74.8	62.3	5.0	No Ab; slight Ag excess (6 μg N)
	37.0	17.0	80.5	63.5	3.7	Ag excess (20 μg N)
	74.0	17.0	77.0	60.0	3.5	Ag excess (57 μg N)
	148.0		73.4			Excess Ag (inhibition)
.25 ml antibeef-albumin serum (No. PC-134) + 1.75 ml. antigen solution						
1.0	.5	.5	9.5	9	18	Excess Ab
	1	1	16	15	15	Excess Ab
	2	2	31	29	14.5	Excess Ab
	4	3.7	51	47.3	12.8	Trace Ab? Trace Ag (.3 μg N)
	8	7	74	67	9.6	No Ab, slight Ag excess (1 μg N)
	16		41.5			Excess Ag, (inhibition)
	32		10			Excess Ag, (inhibition)
	64		5			Excess Ag, (inhibition)
	128		3.5			Excess Ag, (inhibition)
	256		4			Excess Ag, (inhibition)
4.0	.5	.5	12.5	12	24	Excess Ab
	1	1	24.5	23.5	23.5	Excess Ab
	2	2	44	42	21	Excess Ab
	4	4	71	67	16.8	Excess Ab
	8	8	98.5	90.5	11.3	Excess Ab
	16	12.4	108	95.6	7.7	No Ab, slight Ag excess (3.0 μg N)
	32	10.8	94	83.2	7.7	Excess Ag (21.2 μg N), slight inhibition
	64		67.5			Excess Ag, inhibition
	128		39			Excess Ag, inhibition
	256		7.5			Excess Ag, inhibition
8.0	.5	.5	12	11.5	23	Excess Ab
	1	1	23	22	22	Excess Ab
	2	2	43	41	20.5	Excess Ab
	4	4	67	63	15.5	Excess Ab
	8	8	93.5	85.5	10.7	Excess Ab
	16	14	116	102	7.3	No Ab, slight Ag excess (2 μg N)
	32	16.4	116	99.4	6.1	Excess Ag (15.4 μg N)
	64		114			Excess Ag
	128		92			Excess Ag (inhibition)
	256		50			Excess Ag (inhibition)

the increased precipitation in high salt concentration. On the other hand the smaller amount of total antibody and antigen precipitated in the 1% salt shows that with the low salt the precipitation is incomplete. It is also seen that marked inhibition occurred in 1.0% at those antigen concentrations in which maximum precipitation occurred in 4.0 and 8%. The main difference between the results with the 4 and 8% salt as shown in Table III is that with the latter the zone of maximum precipitation extends further into the region of antigen excess. Additional increases of salt concentration cause even greater extensions of this zone (see Table V).

The data in Table III also indicate that at 1% sodium chloride some inhibition of antibody-antigen precipitation occurs even in the presence of sizable quantities of free antibody. This is demonstrated by several different types of results. First of all, when the anti-human-globulin serum was mixed with 4.6 μg N of antigen

TABLE IV

Effect of increasing salt concentration of the supernatants resulting from an antibeef-albumin (PC-134)—beef-albumin reaction; demonstration of free-excess antibody

NaCl conc. of original test	Supernatant salt raised to %	Results	μg beef albumin in 2 ml of original mixture.										
			Cont.	.5	1	2	4	8	16	32	64	128	256
%													
1	8	P*	−	−	−	−	+	+	+	+	+	+	+
		S†	+	+	+	+	+	+	−	−	−	−	−
4	8	P*	−	−	−	−	−	−	−	+	+	+	+
		S†	+	+	+	+	+	+	−	−	−	−	−
8	8	P*	−	−	−	−	−	−	−	−	−	−	−
		S†	+	+	+	+	+	+	−	−	−	−	−

* + = additional antigen-antibody precipitation due to increasing salt concentration to 8%; − = no precipitation.

† Supernatant of 8% reaction tested for antibody by adding antigen. + = free antibody present; − = no free antibody present.

the precipitation in 4 and 8% salt was much greater than in the 1%, for all the antigen was precipitated in the higher salts but not in the lowest one. Secondly, though a negative antibody test was secured with the supernatant of the 1% mixture positive antibody reactions were obtained with the 4 and 8% mixture (these supernatant tests were made in a salt concentration similar to the original). Lastly, the presence of free antibody was further indicated by the similarity of the antibody to antigen ratios of the precipitates formed in the mixtures. The ratios secured at the 4.6 μg N antigen reaction were 11.6, 9.5 and 9.5 respectively in the 1, 4 and 8% salt concentrations. Such high ratios could only occur in the antibody excess zone of this particular system in which the molecular weight of human globulin is about the same as that of chicken antibody (15). Undoubtedly, then, the mechanism that inhibited complete precipitation of antigen with antibody in the 1% salt also prevented the positive test for excess antibody.

The experiments performed with the anti-beef-albumin serum (PC-134) demonstrate even more directly that supernatant tests for antibody carried out in 1% NaCl are misleading. The original data indicate an equivalence at 4 and 8 μg N levels of beef albumin, for the supernatants from these mixtures show no antibody and a small amount of antigen (Table III, col. 7). On the other hand, when the salt concentration of untreated supernatants from duplicate determinations was raised to 8% (by addition of 1 ml of a proper salt solution) additional antibody-

TABLE V

Effect of antiserum dilution on completeness of precipitation in varying salt concentrations—antibeef-albumin chicken serum—beef-albumin system

ANTISERUM NO.	SALT CONC.	ANTISERUM DILUTION*	TURBIDITY MEASUREMENTS (18 HR. READING) IN GALVANOMETER UNITS										
			μG BEEF ALBUMIN IN 2 ML OF ORIGINAL MIXTURE										
			Cont.	.5	1	2	4	8	16	32	64	128	384
	%												
3730–3660	15	1:2	0	5	8	16	26	40†	58†	66	70	75	80
		1:4	0	3	8	12	19†	28†	36	36	39	40	42
		1:8	0	5	7	12†	16†	18	19	21	21	20	20
	8	1:2	0	2	6	10	21	38†	55†	62	68	74	43
		1:4	0	4	5	11	19†	28†	34	35	38	36	3
	4	1:2	0	4	6	12	23	46†	56†	58	44	15	2
		1:4	0	4	6	14	23†	29†	26	15	4	2	—
		1:8	0	—	5	7†	8†	5	1	0	—	—	—
1374–3640	8	1:2	0	4	8	12	23	42†	60†	68	75	76	28
		1:4	0	3	6	12	23†	30†	34	37	38	35	5
		1:8	0	3	6	11†	15†	16	17	17	15	8	—
	4	1:2	0	4	6	12	26	44†	55†	53	40	20	6
		1:4	0	4	5	12	22†	24†	22	19	11	7	—
		1:8	0	2	5	8†	9†	9	5	3	3	3	—
	2	1:2	0	4	5	11	24	36†	31	21	12	6	—
		1 4	0	3	6	12	16†	12	9	7	7	—	—
		1:8	0	2	4	5†	4	3	3	3	3	—	—
	1	1:2	0	4	8	9	19	25†	17	11	8	—	—
		1:4	0	1	6	7	9†	6	5	5	—	—	—
		1:8	0	3	2	2†	1	5	2	2	—	—	—

* 1:2 dilution means 0.15 ml antiserum plus 1.85 ml antigen in saline; 1:4 dilution means 0.075 ml antiserum plus 1.925 ml antigen in saline; 1:8 dilution means 0.0375 ml antiserum plus 1.9625 ml antigen in saline.

† Neutralization zone.

antigen precipitation occurred in those mixtures containing originally 4 or more μg N of antigen. The increased precipitation resulting from the raising of the salt content was separated by centrifugation after overnight standing in the cold room, and the supernatants were now tested for antibody by addition of antigen. Positive tests for antibody were secured in the control tube and in antigens concentrations up to 8 μg N. This clearly shows that no real equivalance zone existed in the reaction run in 1%. Instead some inhibition of antigen-antibody precipitation occurred even in the presence of sizable amounts of free antibody.

The above data are given in Table IV. This table, in addition, records results showing the effect of raising the salt concentration of supernatants to 8% when the original reaction was made in 4%.

The completeness of antibody-antigen precipitation in various sodium chloride concentrations was further studied by antiserum dilution experiments. Table V records such results. The data demonstrate that antiserum dilution adversely affects the precipitation in the lower salt concentrations. When a high salt content such as 8 or 15% was used and the antibody content of the reacting mixture was decreased by 50% (from 1:2 to 1:4 and then 1:8) the turbidimetric readings were decreased by approximately 50% in the region of equivalence and maximum precipitation. On the other hand, a disproportionate decrease in turbidity resulted in lower salt concentrations when the antibody content was lowered. Again

TABLE VI

Effect of salt concentration and dilution of antiserum (antibeef albumin) on speed of precipitation

ANTISERUM NO.	SALT CONC.	ANTISERUM DILUTIONS*								
		1:2			1:4			1:8		
		Turbidity†		Degree‡ of reaction	Turbidity†		Degree‡ of reaction	Turbidity†		Degree‡ of reaction
		1 hr.	18 hrs.		1 hr.	18 hrs.		1 hr.	18 hrs.	
	%			%			%			%
3730–3660	15	410	444	92	233	263	89	146	159	92
	8	311	379	82	168	214	78	—	—	—
	4	185	266	70	50	123	40	5	30	17
1374–3640	8	326	396	82	161	225	72	64	109	59
	4	196	268	73	72	127	57	21	48	44
	2	103	151	65	37	73	51	7	29	24
	1	63	102	62	16	40	40	4	11	36

* 1:2 dilution means 0.15 ml antiserum plus 1.85 ml antigen in saline; 1:4 dilution means 0.075 ml antiserum plus 1.925 ml antigen in saline; 1:8 dilution means 0.0375 ml antiserum plus 1.9625 ml antigen in saline.

† Total galvanometer units for complete precipitation curve.

‡ One hour reading compared to 18 hour reading.

this shows the untrustworthiness of results when low salt concentrations are used in chicken antiserum systems.

The effects of salt concentration and serum dilution on the rapidity of precipitation were also observed. This was done by the photroner method. A comparison of the one hour and overnight precipitation curves was made, and the results are given in Table VI. It can be readily seen that the more concentrated the sodium chloride solution the more rapid is the precipitation during the first hour of incubation. It is also apparent that precipitation becomes more rapid on increasing the concentration of the reacting antiserum.

DISCUSSION

Our experimental results indicate that antibodies produced in chickens against serum proteins exhibit *in vitro* reactions that are very different from those of mam-

malian antisera. It has been observed with mammalian antibodies that when the sodium chloride content is increased above 1% a decreased precipitation results. We have shown, on the other hand, that maximal precipitation of chicken precipitins during a reasonable incubation period occurs in salt concentrations much greater than 1%. In our opinion a salt diluent of at least 8% is necessary to get an accurate quantitative assay of chicken antiserum.

The exact reasons for the incompleteness of the antibody-antigen precipitation in low sodium chloride concentrations cannot as yet be given. However the data suggest the following explanation: From the antibody excess zone to the antigen excess zone and inhibition region antibody-antigen compounds of increasing antigen content and increasing solubility are formed. Even in antibody excess, a portion of these compounds is soluble in a low sodium chloride concentration such as 1%. Then the effect of increasing the salt concentration is that of reducing the solubility of these compounds. Thus certain compounds which, due to their antigen content would be very soluble in 1% sodium chloride, would be almost completely insoluble in a higher NaCl concentration such as 8%.

An alternative explanation, one postulating a high dissociation of the antibody-antigen compounds in low salt concentration, is also suggested by the data. The primary effect of increasing the sodium chloride concentration would then be to reduce this dissociation and thereby cause greater precipitation.

The whole problem of the behavior of chicken precipitins cannot be solved until much additional information is obtained. Therefore the effects on chicken precipitins of temperature, incubation time, various ions, pH and other factors are now being studied.

SUMMARY

1. The effect of varied NaCl concentrations on the reaction of chicken antisera with serum protein antigens was studied by turbidimetric and quantitative nitrogen techniques. The results of the two methods paralleled each other closely.

2. Increasing the NaCl concentration up to that range in which there was some salting out of chicken serum proteins (14–18%) resulted in marked increases of precipitation with the maximum occurring at progressively higher antigen concentrations.

3. The reaction was solely between specific antigen and antibody, and the effect of increasing the salt content was to cause a more complete precipitation of this antigen and antibody.

4. Thus determinations of maximum antibody content could not be made at salt concentrations of less than about 8%.

5. The precipitation also became more rapid with increases of salt concentration and reacting antibody.

6. The significance of the above results was discussed.

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