the seven monohaptenic substances failed to precipitate. It is pointed out that these results provide strong support of the framework theory of the precipitin reaction.

Data on the amounts of precipitate formed are discussed in relation to the structure of the simple antigens.

PASADENA, CALIFORNIA RECEIVED JULY 6, 1942

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 886]

The Serological Properties of Simple Substances. II. The Effects of Changed Conditions and of Added Haptens on Precipitation Reactions of Polyhaptenic Simple Substances

BY LINUS PAULING, DAVID PRESSMAN, DAN H. CAMPBELL, AND CAROL IKEDA

During the course of the investigation of precipitation reactions of polyhaptenic simple substances reported in the preceding paper of this series[1] we found it desirable to carry out a study of the effects of changed conditions of precipitation and washing on the amount of residual precipitate. We also made some experiments on the inhibition of precipitation by added haptens, in order to see how great would be the effects of monohaptenic impurities possibly present in the substances studied. The results obtained are presented and discussed in this paper.

Experimental Methods.—The experiments were carried out in the way described in the preceding paper (I). In addition to antisera C and D mentioned in paper I, three antisera, E, F, and G, were used. E and G contained amounts 0.6 and 3.2 mg. per ml., respectively, of antibody precipitable by azo-ovalbumin test antigen; the strength of F was not determined.

The borate buffer solutions were made by adding suitable amounts of 0.16 N sodium hydroxide solution to 0.2 M boric acid solution containing 0.9% sodium chloride.

The Effect of Changed Conditions of Precipitation and Washing on the Amount of Precipitate.—It is seen from the data reported in Tables I, II, and III, obtained with two antigens (VI and X) and three antisera (C, E, and F), that the antigen-antibody precipitate is either dissolved slightly or carried away mechanically by the saline or borate buffer solutions with which it is washed. The loss in this way is, however, small, amounting to about 5 to 15% for eight or ten extra washings with 10-ml. portions of solution.

A few experiments were made (Tables II and III) to test the effects of changing the time and temperature of precipitation. It was found that increasing the precipitation time from one day to two days increases the amount of precipitate by about 10%. The amount of precipitate formed seems to increase with increase in temperature of

TABLE I

EFFECT OF NUMBER OF WASHINGS ON AMOUNT OF RESIDUAL PRECIPITATE

3 ml. antigen VI, 25 μg./ml. in saline solution, plus 3 ml. antiserum C						2 ml. antigen VI plus 2 ml. antiserum E		
	Composition of precipitate			Composition of precipitate			Composition of precipitate	
Washings	Antibody[a]	Antigen[a]	Washings	Antibody[a]	Antigen[a]	Washings	Antibody[a]	Antigen[a]
2	1665	8.9	6	1600	8.7	3	1110	5.7
	1660	8.4		1540	8.7		1125	6.2
	1660	8.9		1590	8.7	10	970	5.7
3	1600	8.7	7	1590	8.7		930	6.0
	1600	8.9		1530	8.9	15	960	6.0
	1545	8.7		1510	8.9			
4	1545	8.7	8	1510	9.2			
	1570	8.9		1530	8.7			
	1660	8.7		1480	8.1			
5	1570	8.9	10	1520	9.5			
	1570	8.9		1510	7.6			
	1570	9.5		1320	9.5			

[a] Amounts of precipitated antibody and antigen in micrograms. pH of all supernates 8.1.

(1) Linus Pauling, David Pressman, Dan H. Campbell, Carol Ikeda, and M. Ikawa, THIS JOURNAL, **64**, 2994 (1942). We shall refer to this as paper I.

TABLE II

EFFECT OF CHANGED CONDITIONS ON AMOUNT OF PRECIPITATE

3 ml. antigen VI, 12.5 μg./ml., in saline solution, plus 3 ml. antiserum E. *p*H of supernates 8.0 to 8.3.

Conditions of precipitation	Washings	Composition of precipitate: Antibody, μg.	Antigen, μg.
Room 1 hr., refrigerator	3	1600	9.2
24 hr.		1610	8.9
	10	1190	8.4
		1310	8.1
		1140	8.7
	15	1140	9.2
Room 1 hr., refrigerator		1780	9.2
48 hr.	3	1590	8.9
Refrigerator 24 hr.	3	1590	8.9
		1640	9.2
Refrigerator 48 hr.	3	1610	8.9
		1690	8.9
Room 24 hr.	3	1080	7.0
		980	7.0

TABLE III

EFFECT OF CHANGED CONDITIONS ON AMOUNT OF PRECIPITATE

5 ml. antigen X, 20 μg./ml. in saline solution, 2.5 ml. antiserum F, and 7.5 ml. borate buffer of *p*H 9.0.

Conditions of precipitation	Washing solution	Washings	Composition of precipitate: Antibody, μg.	Antigen, μg.
Room 1 hr., refrigerator 24	Saline	3	520	3.3
			520	3.3
hr.	Saline	10	525	3.1
			530	3.1
	Saline, iced	3	585	3.3
			520	2.9
	Buffer,	3	600	3.3
	Buffer, iced	3	630	2.9
			600	2.9
Room 1 hr., refrigerator 48 hr.	Saline	3	580	2.9
			595	2.9
Room 3 hr., refrigerator 22 hr.	Saline	3	610	3.1
			550	3.1
Room 24 hr.	Saline	3	675	3.1
			605	3.1
35° 1 hr., refrigerator 24 hr.	Saline	3	560	2.9
			555	2.9

the tube during the first few hours of the precipitation period (Table III). However, the evidence is inconclusive as to whether the final amount of precipitate is increased or not by refrigeration during the later part of the precipitation period.

The data reported in Table IV show that the amount of precipitated antibody is decreased by the addition of buffer solution to the antigen-antibody mixture. The decrease is not proportional to the volume of buffer added, so that the phenomenon is not analogous to the solution of a

TABLE IV

THE EFFECT OF DILUTION WITH BUFFER SOLUTION ON AMOUNT OF PRECIPITATED ANTIBODY

2.5 ml. antigen VI, varying volume of borate buffer solution with *p*H 8.0, 2.5 ml. antiserum C.

Volume of buffer solution	Amount of antigen used (μg.): 15.6	31.3	62.5
	Amount of precipitated antibody (μg.)		
0	750	1375	775
2.5	600	1030	990
5	480	845	525
10	470	655	450

well-defined compound. These observations provide further evidence of the heterogeneity of the antibodies in immune sera.

The Effect of Hydrogen-ion Concentration on Amount of Precipitate.—The results of experiments to test the effect of change in *p*H on the amount of precipitate are given in Table V. It is seen that for this antigen-antibody system the optimum *p*H is about 8.1, the amounts of precipitate in this region being greater than for either more acidic or more basic solutions. Evidence that the buffering substances do not have a large direct influence on the reaction is given by the agreement of the values at *p*H 8.1 for added saline solution and added borate buffer.

The optimum antigen concentration is seen to be changed only slightly by change in *p*H.

Less extensive experiments were also carried out with the amide antigen XIV (R″–R″) in place of the azo antigen; the results obtained, given in Table VI, are similar.

The Effect of Dilution with Normal Serum or Buffer Solution.—In order to determine the effect of change in the strength of a serum of fixed antibody composition on the position of the optimum zone, identical experiments were made with sera obtained by mixing antiserum G and normal serum. The results are given in Table VII. It is seen that for both antigens (III and VI) the maximum precipitation occurs at an amount of antigen about midway between 25 and 50 μg. (for 2 ml. of antiserum), and that on two-fold dilution of the antiserum, decreasing its antibody concentration by the divisor 2, the optimum amount of antigen is also decreased by about the divisor 2. The effect of diluting the antiserum is hence to cause the optimum zone to shift in such a way as to keep constant the ratio of antigen to antibody.

The same result is given by experiments on the effect of dilution with buffer solution, reported

TABLE V

EFFECT OF HYDROGEN-ION CONCENTRATION ON AMOUNT OF PRECIPITATE

3 ml. saline solution VI, 3 ml. antiserum E, and 3 ml. saline or buffer solution; 48 hrs. in refrigerator. B = antibody in precipitate, micrograms. A = antigen in precipitate, micrograms.

Amount of antigen (μg.) Added solution	pH of supernate	22.2 B	22.2 A	33.3 B	33.3 A	50 B	50 A	75 B	75 A
Boric acid	7.6–7.7	640	4.9	820	5.9	820	5.7	610	4.1
		640	4.6	820	5.7	820	5.7	620	5.4
Saline	8.1–8.2	890	4.6	1140	6.5	1020	6.2	610	3.0
		840	4.6	1140	6.5		6.2	550	3.8
Borate buffer,	8.1	820	5.7	1080	8.4	940	7.0	650	4.9
*p*H 8.0		790	5.7	1080	7.3	920	6.0	590	5.4
Borate buffer,	8.8–8.9	680	5.1	780	5.9	640	6.2	520	5.4
*p*H 9.0		650	4.6	820	6.5	720	6.2	520	4.3

TABLE VI

EFFECT OF HYDROGEN-ION CONCENTRATION ON AMOUNT OF PRECIPITATED ANTIBODY

1 ml. antigen XIV, 1 ml. antiserum G, 2 ml. saline or buffer solution.

Amount of added antigen (μg.) Added solution	pH of supernate	12.5 Amount of precipitated antibody (μg.)	25 Amount of precipitated antibody (μg.)	50 Amount of precipitated antibody (μg.)
Boric acid	7.6	200	275	260
		250	290	240
Saline	8.0–8.2	270	305	270
		290	345	265
Borate buffer,	8.1	270	290	225
*p*H 8.0		290	375	325
Borate buffer,	8.8–9.0	215	290	240
*p*H 9.0		280	295	270

TABLE VII

THE EFFECT OF DILUTION WITH NORMAL SERUM ON AMOUNT OF PRECIPITATED ANTIBODY

3 ml. of antigen III or VI plus 2 ml. of mixture of normal serum and antiserum G.

Amount of antigen used (μg.) Antigen	Antiserum	Normal serum	12.5 Amount of precipitated antibody (μg.)	25 Amount of precipitated antibody (μg.)	50 Amount of precipitated antibody (μg.)	100 Amount of precipitated antibody (μg.)
III	2	0	230	495	495	265
			215		555	230
	1	1	305	340	200	105
			305	280	215	55
	0.5	1.5	225	170	105	10
			230	170	105	0
VI	2	0	395	1150	1070	170
			400	1100	1240	170
	1	1	495	455	195	65
			425	510	205	70
	0.5	1.5	290	120	40	0
			330	200	45	0
*p*H of supernate	antigen III		8.1	8.1	8.1	8.1
	antigen VI		8.2	8.3	8.4	8.5

in Table IV. In these experiments, in which the amount of antibody is kept constant, the position of the optimum zone does not change significantly.

These conclusions agree with those reached by many earlier investigators with protein antigens.

The Inhibition of Precipitation by Hapten.—It was discovered by Landsteiner[2] that a hapten such as arsanilic acid present in reasonable concentration in a mixture containing an azoprotein (made from this hapten) and the hapten-homologous antiserum decreases the amount or inhibits the formation of the antigen-antibody precipitate. It has also been found[3] that this phenomenon occurs with polyhaptenic simple substances replacing the azoprotein. Quantitative results were obtained by us with antigens VI, X, and XX, containing the groups R, R′, and R″, respectively, and haptens XXI, XXII, XXIII, and XXVII. These are given in Tables VIII to XI and Figs. 1 and 6. It is seen (Table VIII) that addition of hapten decreases the amount of precipitate without noticeable shift in the equivalence zone, and (Tables X, XI) that haptens differ in their inhibiting power.

TABLE VIII

THE INHIBITION OF PRECIPITATION BY HAPTEN

3 ml. antigen VI, 1 ml. hapten XXI, 3 ml. antiserum E. *p*H of supernates 8.2.

Amount of antigen (μg.) Amount of hapten (μg.)	15 Amount of precipitated antibody (μg.)	22.2 Amount of precipitated antibody (μg.)	31.3 Amount of precipitated antibody (μg.)	50 Amount of precipitated antibody (μg.)	75 Amount of precipitated antibody (μg.)
0	540	790	1280	1020	560
12.5	520	740	910	850	540
25	520	580		780	530
50	340	560	630	640	530
100	330	360	610	550	450
200	290	340	430	440	360
400	200	270	290		270

TABLE IX

INHIBITION BY HAPTEN

1 ml. antigen VI (12.5 μg.), 0.5 ml. hapten XXIII, 1 ml. antiserum D. *p*H of supernates 8.1.

Amount of hapten, μg.	Amount of precipitated antibody, μg.
0	505 470
3.13	505
6.25	480
12.5	355
25	220
50	70

(2) K. Landsteiner, *Biochem. Z.*, **98**, 117 (1919); **104**, 280 (1920); K. Landsteiner and J. van der Scheer, *J. Exptl. Med.*, **48**, 315 (1928); **50**, 407 (1929); **54**, 295 (1931); **55**, 781 (1932).

(3) K. Landsteiner and J. van der Scheer, *ibid.*, **56**, 399 (1932).

TABLE X

INHIBITION BY HAPTEN

0.5 ml. antigen X (25 μg.), 0.2 ml. hapten XXII or XXIII, 0.5 ml. antiserum D.

Amount of hapten, μg.	Amount of precipitated antibody, μg. Hapten XXII	XXIII
0	570	565
1.25	505	565
2.50	495	530
5	420	390
10	455	315
20	295	125
40	210	65
*p*H of supernates	8.5	8.8

TABLE XI

INHIBITION BY HAPTEN

1 ml. antigen XX (25 μg.), 0.5 ml. hapten XXII or XXVII, 1 ml. antiserum D.

Amount of hapten, μg.	Amount of precipitated antibody, μg. Hapten XXII	XXVII
0	1305	1200
3.13	1295	1120
6.25	1225	845
12.5	1075	590
25	960	215
50	650	
100	475	40
*p*H of supernates	8.2	8.3

The results obtained show that no significant error would be introduced in the experiments by the presence of haptens as impurities in the polyhaptenic antigens in amounts as great as 5%. It is improbable that any of the substances used contained this much monohaptenic impurity.

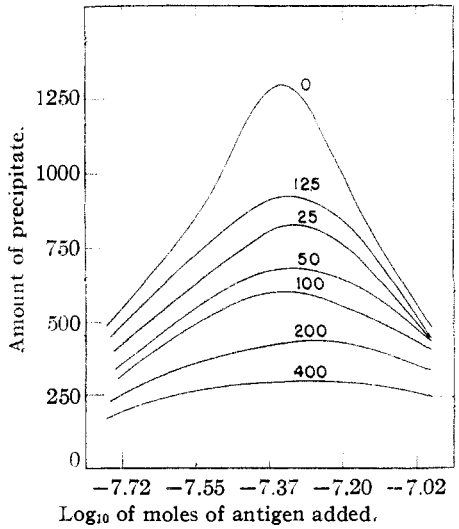


Fig. 1.—Effect of added hapten (in amounts given) on amount of antigen-antibody precipitate (Table VIII).

The data reported in Table XII indicate that the same final equilibrium is reached by the system antigen-antibody-hapten when the order of combining the reactants is changed.

TABLE XII

EFFECT OF ORDER OF COMBINATION OF REACTANTS

Reactants combined as indicated and allowed to stand indicated times at room temperature, then 24 hours in refrigerator: A, 3 ml. solution of antigen VI (37.5 μg.); H, 1 ml. solution of hapten XXI (100 μg.); S, 3 ml. antiserum E. *p*H of supernates 8.1.

	Amount of precipitated antibody, μg.	
1. A + S + 1 ml. saline solution $1^1/_2$ hours	1200	1090
2. A + S + H, $1^1/_2$ hours	520	520
3. S + H, $^1/_2$ hour, + A, 1 hour	550	525
4. A + S, $^1/_2$ hour, + H, 1 hour[a]	550	520
5. Same as 4, with shaking.[a]	550	525

[a] A precipitate formed before hapten was added.

Discussion

A reasonable interpretation of these results and those of the preceding paper can be given in terms of the multivalent-antibody theory. This interpretation is conveniently presented with the aid of a simplified model susceptible to easy mathematical treatment.[4]

Let us assume that our idealized antigen–antibody system consists of a solution containing antigen molecules A, antibody molecules B, soluble complex molecules A_2B, and molecules AB in equilibrium with a precipitate AB. We ignore other complexes A_3B_2, A_4B_3, AB_2, etc., and the known heterogeneity of antibody molecules in a serum.

For simplicity we assume that each of the two bonds in A–B–A is equal in strength to the bond in A–B, and that the equilibrium constants for the two reactions

$$A + B = AB$$

and

$$A + AB = A_2B$$

differ only by the entropy factor 4. We represent these by $4K$ and K, respectively, with K the equilibrium constant for combination of a single haptenic group of an antigen molecule and a single complementary region of an antibody molecule, and derive the equation

$$AB(\text{pp}) = A_{\text{total}} - s - \frac{1 + 2Ks}{2 + 2Ks}\{A_{\text{total}} - B_{\text{total}} + [s(1 + Ks)/K + (A_{\text{total}} - B_{\text{total}})^2]^{1/2}\} \quad (1)$$

(4) Somewhat similar quantitative theories of the precipitin reaction have been published by M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, **61**, 563, **62**, 467, 697 (1935); **66**, 229 (1937); F. E. Kendall, *Annals N. Y. Acad. Sci.*, 153, 85 (1942); and A. D. Hershey, *J. Immunol.*, **42**, 455 (1941). These theories are designed to apply more broadly than ours, which is based on postulates suited to the special antigens and haptens which we are studying.

in which AB (pp) is the amount of precipitated compound, with solubility s, and A_{total} and B_{total} are the total amounts (per unit volume) of antigen and antibody in all molecular species, including the precipitate.

The curves of amount of precipitate for a given antiserum with varying amounts of antigen calculated with this equation have the general shape indicated by the experimental points.

Curves for the arbitrary values $s = 1$, $K = 1/2$ are plotted against A_{total} in Figure 2 for each of several values of B_{total}, corresponding to the strength of the serum. It is seen that in each case the maximum amount of precipitate is produced by an amount of antigen approximately equal to the amount of antibody. This is in agreement with the results obtained with diluted serum (Tables IV and VII).

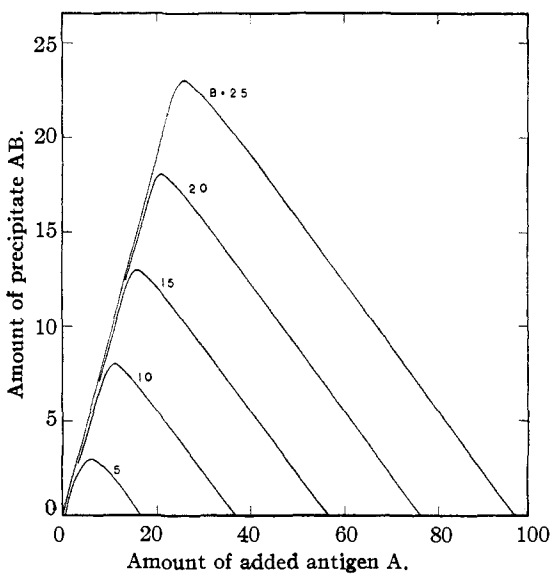


Fig. 2.—Theoretical curves showing amount of precipitate AB as function of amount of antigen A for antisera with varying antibody concentration $B = 5$ to 25. Values of constants used are $s = 1$, $K = 1/2$.

The observation, reported in the preceding paper, that different polyhaptenic antigens containing the same haptenic group have the same molal concentration for maximum precipitation with a given serum, although the amount of precipitable antibody in the serum varies with the antigen, requires explanation, since it might well be expected that the optimum antigen concentration would be proportional to the amount of precipitate. Let us assume that for antigens containing the same haptenic group the A–B bond constant K has the same value, but that the solubility s of the precipitate may vary. This might reasonably result from steric interference of the large antibody molecules in the chains -A–B–A–B–A–B- in the precipitate, which might cause a second bond formed by a bivalent antigen molecule to be much weaker than the first bond. The curves in Fig. 3, with K constant and s varying, represent this situation. We see that, as the result of the consecutive equilibria A + B = AB and AB + A = A_2B, the position of the maximum is constant when K is constant and the solubility s varies. It is found from the equation, in fact, that the maximum occurs at the point $A_{total} - B_{total} = 1/2K$, and is independent of s.

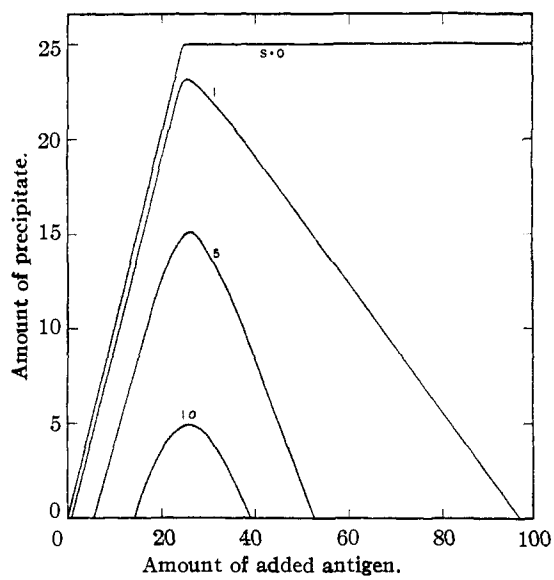


Fig. 3.—Calculated effect of variation of solubility of antigen-antibody precipitate on amount of precipitate; all curves for initial antibody concentration $B = 25$ and $K = 1/2$.

It is also found (Fig. 4) that variation in K produces little change in the optimum antigen concentration, except when the value of K becomes very small. It accordingly seems probable that the difference of the optimum concentration for antigens containing the haptenic group R and those containing the longer group R′, as shown in Fig. 3 of paper I, is evidence that the effective strength of the serum for groups R′ is greater than that for R, because of the presence of antibodies capable of combining with R′ and not with R.

The maximum amount of precipitate is independent of K; its value, as found from Equation 1, is $B_{total} - 2s$.

The fact that change in effective strength of a serum with change in pH is not accompanied by

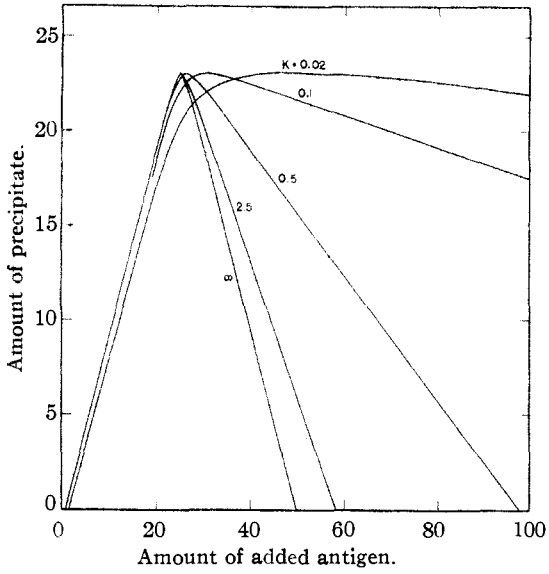


Fig. 4.—Calculated effect of variation of A–B bond-strength constant K on amount of precipitate; all curves for initial antibody concentration $B = 25$ and AB solubility $s = 1$.

shift of the optimum antigen concentration indicates that the effect is not due simply to change in the concentration of effective antibody molecules. Further experiments on the *p*H effect are under way.

The phenomenon of hapten inhibition has been explained by Landsteiner as resulting from combination of hapten and antibody to form a soluble complex, thus effectively neutralizing the antibody. The formation of soluble complex instead of a precipitate by antibody and hapten is explained by the framework theory as the result of the univalence of the hapten. It might be expected that as the maximum amount of precipitate which can be obtained from a serum is decreased by addition of hapten there would occur a corresponding decrease in the optimum antigen concentration, as was observed in the dilution experiments.

It is seen from Fig. 1, however, that the optimum antigen concentration is not shifted very much by addition of hapten. A small shift can be predicted by an extension of our simple theory. If we consider a system containing in solution the molecular species H, BH, BH_2, and ABH (H = hapten) as well as A, B, AB, and A_2B, and assume the B—H bond strength to be such that the equilibrium constants for the reactions

$$B + H = BH$$
$$BH + H = BH_2$$

and

$$AB + H = ABH$$

are $2K'$, $K'/2$, and K', respectively, we obtain the set of equations

$$AB(\text{pp}) = A_{\text{total}} - s - \alpha\{1 + 2K(s + z)\} \quad (2)$$

$$A_{\text{total}} - B_{\text{total}} = \alpha - \frac{s}{4K\alpha} + \alpha Ks - z - \frac{\alpha K z^2}{s} \quad (3)$$

$$z = -\frac{1}{2}\left(\frac{s}{2K\alpha} + s + \frac{1}{K'}\right) \pm \left\{\frac{1}{4}\left(\frac{s}{2K\alpha} + s + \frac{1}{K'}\right)^2 + \frac{sH_{\text{total}}}{2K\alpha}\right\}^{1/2} \quad (4)$$

in which H_{total} is the total hapten concentration. The auxiliary variables α (the concentration of the molecular species A in solution) and z (the concentration of the molecular species HB in solution) are related by Equation 4, by means of which z can be calculated for an assumed value of α. Then by use of Equation 2 the amount of precipitate can be found, and by Equation 3 the variable α can be replaced by A_{total} and B_{total}.

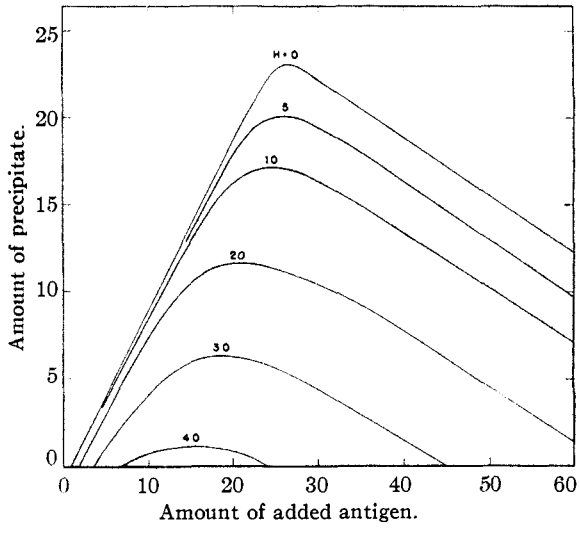


Fig. 5.—Calculated effect of addition of hapten on amount of antigen-antibody precipitate, for $B_{\text{total}} = 25$, $K = 1/2$, and $K' = s = 1$.

In Fig. 5 there are shown curves calculated in this way for $K = 1/2$, $K' = s = 1$, $B_{\text{total}} = 25$, and $H_{\text{total}} = 0$, 5, 10, 20, 30, and 40. It is seen that there is a small shift of the maxima toward lower antigen concentrations. In the region of the equivalence zone, where A_{total} equals B_{total}, the amount of precipitate formed is proportional to the hapten concentration, as is given by Equation 5, which is derived from Equations 2, 3, and 4.

$$\frac{dAB(\text{pp})}{dH_{\text{total}}} = -\frac{1/2 + Ks + (K^2s^2 + Ks)^{1/2}}{1 + Ks + \left(1 + \frac{1}{K's}\right)(K^2s^2 + Ks)^{1/2}} \quad (5)$$

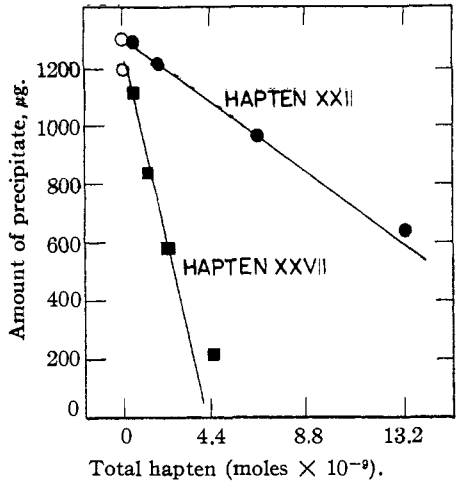
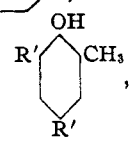


Fig. 6.—Observed effect of haptens XXII and XXVII on amount of precipitate between antigen XX and antiserum (Table XI).

The experimental data at low hapten concentrations are in rough agreement with the straight-line relation, as shown in Fig. 6 for the data of Table XI.

In Fig. 7 there is shown the predicted effect of variation of the hapten-antibody bond-strength constant K'.

It might be expected from its similarity in structure with the antigen that hapten XXVII, NH_2⬡R'', would be much more effective than hapten XXII, arsanilic acid, in inhibiting precipitation by antigen XX, R''⬡R''; that this expectation is borne out can be seen from the slopes of the curves of Fig. 6. The data of Table X show that hapten XXIII, HO⬡R, has greater inhibiting effect for antigen X,



than has hapten XXII.

The data of Tables VIII and IX cannot be interpreted so reliably, since the antigen used (VI) is trihaptenic. The observation that hapten XXIII is very much more effective than hapten XXI, phenylarsonic acid, is however to be expected from the structures. We are planning to continue work on hapten inhibition, with the hope of obtaining quantitative information about the relative bond strengths of different haptenic groups with antibody.

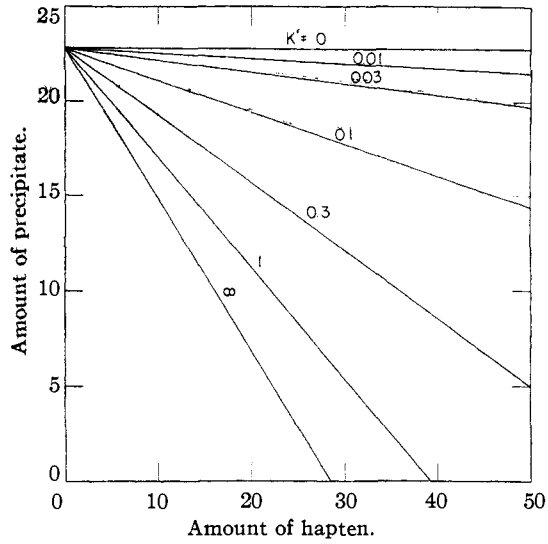


Fig. 7.—Calculated dependence of amount of antigen-antibody precipitate on hapten-antibody bond-strength constant K', for $A_{total} = B_{total} = 25$, $K = 1/2$, $s = 1$.

We thank the Rockefeller Foundation for financial support of this work. We are indebted also to Dr. Verner Schomaker for helping with the theoretical treatment of antibody-antigen-hapten interactions, and to Mr. Shelton Steinle for carrying out analyses.

Summary

The results are reported of experimental studies of the effect of changed conditions, including time and temperature of precipitation, washing, addition of buffer solution and normal serum, hydrogen-ion concentration, and addition of hapten, on amount of precipitate formed by antisera and simple polyhaptenic antigens of known structure.

A simple theory of antibody-antigen-hapten interaction is formulated on the assumption of the bivalence of antibodies. It is found that this theory provides a reasonable interpretation of the experiments.

PASADENA, CALIFORNIA RECEIVED JULY 6, 1942