bromine atoms is responsible for the greater difference in the moments of the dibromides as compared with the dichlorides. The fact that the *dl*-stilbene dibromide has a moment higher than that to be expected for a random distribution about the ethane C–C link, again appears to be the result of London forces between the bromine atoms.[8,9]

The electric moment of *diacetyl* was calculated by Zahn[10] from the moments of the component groups. Assuming a random distribution around the C–C linkage (free rotation), the moment should be $3.2D$. The same author found, experimentally, a moment which varies between 55° and 230° from 1.2_5 to 1.4_8D. These values and the temperature dependence itself indicate a considerable mutual interference of the two acetyl groups. With acetyl acetone, where a CH_2 group is inserted between the acetyl groups, complications arise from enolization.[10]

In *p*-diacetylbenzene the acetyl groups are separated by a benzene nucleus *p*-Diacetylbenzene was prepared according to the method of Berend and Herms.[11] It had a m. p. of 113–114°. The results of Sängewald's measurements and calculations are given in the table.

The electric moment of *p*-diacetylbenzene, 2.7_1D, is much closer to that calculated for diacetyl than the moment of diacetyl itself. However, it is still about 15% lower than the calculated value. According to Smyth,[12] resonance may give sufficient double bond character to the bond between the acetyl group and the ring to favor *cis*- and *trans*-forms. The observed moment would then depend upon the relative stability of the two forms.

Attempts by Sängewald to measure the moment of 4,4'-diacetyldiphenyl were frustrated by the low solubility of the compound.

(9) G. C. Hampson and A. Weissberger, THIS JOURNAL, **58**, 2111 (1936).

(10) C. T. Zahn, *Physik. Z.*, **34**, 570 (1933).

(11) W. Berend and P. Herms, *J. prakt. Chem.*, [2] **74**, 134 (1906).

(12) C. P. Smyth, private communication.

TABLE I

MEASUREMENTS IN BENZENE

f_2, %	d_4^t	ϵ	n^2	$P_{1,2}$	$P''_{1,2}$
α-Stilbene dibromide, t = 20.0 ± 0.1°					
0	0.878825	2.28400		26.6173	
0.00918	.878985	2.284325		26.6241	
.01578	.879105	2.284166		26.6254	
$dP_{1,2}/df_2 \sim 70$, P_2 = 90 ± 10, P_2'' calcd. = 76, μ = 0.4 − 0.9D					
β-Stilbene dibromide, t = 25.0 ± 0.1°					
0	0.8735	2.272	2.2443	26.610	26.194
0.3004	.8782	2.311	2.2476	27.298	26.366
.4122	.8803	2.320	2.2482	27.463	26.409
.5331	.8828	2.340	2.2497	27.786	26.464
$dP_{1,2}/df_2$ = 216, $dP_{1,2}''/df_2$ = 53.4, P_2 = 243, P_2'' = 79.6, μ = 2.8$_1D$					
Diacetylbenzene, t = 25.0 ± 0.1°					
0	0.8731	2.282	2.2427	26.763	26.184
0.6294	.8757	2.339	2.2446	27.688	26.310
0.9624	.8771	2.383	2.2455	28.368	26.377
1.325	.8786	2.433	2.2473	29.126	26.460
$dP_{1,2}/df_2$ = 172, $dP_{1,2}''/df_2$ = 20.8, P_2 = 199, P_2'' = 46.9, μ = 2.7$_1D$					

Summary

The electric moments of the stilbene dibromides and of *p*-diacetylbenzene are determined and discussed.

It is concluded from the moments of the stilbene dibromides that α-stilbene dibromide is the *meso*-compound and β-stilbene dibromide is the *dl*-compound.

ROCHESTER, N. Y. RECEIVED FEBRUARY 24, 1945

[CONTRIBUTION FROM SEROLOGICAL LABORATORY, UNIVERSITY HOSPITAL, UNIVERSITY OF MICHIGAN]

The Effect of Haptens on Serological Precipitates, with Experimental Data on Kahn Precipitates

BY J. DAVID NEWBURGH

Theoretical Considerations

Various methods have been suggested to describe the amount of antigen–antibody precipitate in terms of the amounts of antigen and antibody present. The method used here, *i. e.*, the use of the fundamental laws of equilibrium, has recently been advocated by Pauling and others.[1] For definiteness, we consider antigen molecules, A, which have two regions, each of which is capable of forming specific bonds with the antibody, B. The antibody is also considered bivalent. The combination of the antibody with one of these two groups of the antigen is taken to be independent of whether or not the other group is free or combined with an antibody molecule. H represents a monovalent hapten, *i. e.*, it has one group capable of being bonded to the antibody. The composition of the precipitate will be $(AB)_n$, n being a large number; its amount is represented by AB_{pp} and its solubility by s. The total amounts of antigen, antibody, and hapten will be indicated by A_t, B_t, and H_t, respectively. For convenience, several concentrations are abbreviated as follows:

$[A] = \alpha$	$[B] = \beta$	$[H] = \eta$
$[AB] = s$	$[A_2B] = a$	$[AB_2] = b$
$[BH] = h$	$[BH_2] = k$	

(1) L. Pauling, D. Pressman, D. H. Campbell and C. Ikeda, THIS JOURNAL, **64**, 3003 (1942); and Pauling, Campbell and Pressman, *Physiol Rev.*, **23**, 203 (1943).

There will then be five independent equilibria, each with a corresponding mass law equation.

$$\begin{aligned} A + B &\rightleftarrows AB & s/\alpha\beta &= 8K & (1)\\ A + AB &\rightleftarrows A_2B & a/\alpha s &= 2K & (2)\\ B + AB &\rightleftarrows AB_2 & b/\beta s &= 2K & (3)\\ B + H &\rightleftarrows HB & h/\beta\eta &= 4K' & (4)\\ H + HB &\rightleftarrows H_2B & k/h\eta &= K' & (5) \end{aligned}$$

The relations implied between the equilibrium constants are first approximations based upon consideration of the symmetry of the species involved. Their accuracy is not essential to the theory.

The total amounts of antigen, antibody, and monovalent hapten are given by summation

$$AB_{pp} + s + \alpha + 2a + b = A_t \quad (6)$$

$$AB_{pp} + s + \beta + a + 2b + h + k = B_t \quad (7)$$

$$\eta + h + 2k = H_t \quad (8)$$

The relations (1) through (5) may be solved for β, η, a, b, and k in terms of α and h, and the constants s, K, and K'. These results are then substituted into (6), (7), and (8) to give

$$AB_{pp} + s + \alpha + 4K\alpha s + s^2/4\alpha = A_t \quad (9)$$

$$AB_{pp} + s + s/8K\alpha + 2K\alpha s + s^2/2\alpha + h + 2K\alpha h^2/s = B_t \quad (10)$$

$$2K\alpha h/K's + h + 4K\alpha h^2/s = H_t \quad (11)$$

If A_t, B_t, and H_t are given, this is a system of three equations in the unknowns AB_{pp}, α, and h, and serves to determine AB_{pp}. On the other hand, AB_{pp} and B_t may be given arbitrary values, in which case the equations imply a relation between A_t and H_t. For fixed B_t, the relations may be represented by a series of contours of constant AB_{pp} in the A_t–H_t plane. Of particular interest is the curve for $AB_{pp} = 0$, since for any experiment represented by a point enclosed by the curve and the A_t axis, precipitation occurs, whereas for a point on the other side of the curve, no precipitation occurs. That AB_{pp} is always positive on the same side of the curve $AB_{pp} = 0$ follows from the single-valuedness of AB_{pp} as a function of A_t, B_t and H_t, which implies that contours for differing values of AB_{pp} do not intersect. The known region of precipitation for $H_t = 0$ shows that precipitation must occur on the inside of the curve $AB_{pp} = 0$.

Accordingly, a curve has been plotted for $AB_{pp} = 0$, and the arbitrary values $s = {}^1/_{10}$, $B_t = 1$, $K = K' = 10$. The equivalence of K and K' implies that the strength of the A–B bond is approximately equal to that of the H–B bond. It is apparent that two well-known phenomena are represented by the curve in Fig. 1. For $H_t = 0$, a zone of precipitation is shown, that is, in order to get precipitation the amount of antigen must be neither too large nor too small. Secondly, a sufficient amount of hapten will completely prevent precipitation, no matter what the amount of antigen. It is therefore not surprising that near the antigen-excess end of the zone, the addition of a small quantity of hapten will inhibit the formation of a precipitate. A pair of experiments illustrating this are indicated by X and X'.

The action of a small amount of hapten at or just beyond the antibody-excess end of the zone is of more interest. It is clear from the curve that the presence of the hapten will result in the formation of a precipitate with an amount of antigen too small to give a precipitate in the absence of hapten. Y and Y' illustrate this phenomenon. Also, for amounts of antigen small enough to give only weak precipitates, the addition of hapten should result in more pronounced precipitation.

This same phenomenon should also be expected on the basis of somewhat cruder reasoning. The lack of precipitation at the antibody-excess end of the zone is believed to be due to the formation of soluble complexes containing more antibody than the precipitate. Hence removal of some of the antibody by combination with the hapten would be expected to favor precipitation. A difficulty here is that the hapten will also form complexes with the antibody which will still be effective in saturating the combining groups of the antigen but are incapable of forming a precipitate. Thus BH would be as effective in rendering A ineffective for precipitation as B, by forming HBABH instead of BAB, but BH could not form long chains with A, as could B.

There are certain possible objections to using the equations (1) through (8) to represent serological systems. In the first place, actual systems are certainly not so simple. The number of combining groups of both antigen and antibody was assumed to be two. Although this may be close for the antibody, it probably holds for only a few antigens. However, this is not serious, since it would be possible to make modifications to conform to any definite valencies. But actual antigens and antibodies may well be mixtures of types containing several different valencies in unknown amounts. In the case of the antibody, at least, the actual combining groups themselves are not all alike; antibodies are characteristically heterogeneous in this respect. However, none of these objections would prevent us from regarding equations (1) through (8) as a useful and conceivable theoretical model of actual systems, with many important properties in common with them.

A further difficulty is that the selection of the combinations of A, B, and H to be considered was arbitrary. Thus from the nature of the forces involved, there seems to be no reason to exclude ABH from consideration, but to consider both ABA and HBH. Actually it is easy to carry through the calculation including ABH, and the result is not changed in any essential way. But extension of the calculations would soon become prohibitively laborious, and other complications would arise. For instance, in the series $(AB)_nA$ with n large enough, the solubility would become small enough so that the possibility of precipitation would have to be considered, and this would require some change in method. Therefore, the system as presented cannot be considered a com-

TABLE I[a]

INHIBITORY EFFECT OF CHOLINE

Antigen suspension, cc.		0.005	0.010	0.020	0.040	0.080
Diluted serum, cc.		0.1	0.1	0.1	0.1	0.1
Serum A	Control	4	4	4	4	–
	Serum (0.5 cc.) + choline (0.5 g.)	–	–	–	–	–
Serum B	Control	4	4	4	–	–
	Serum (0.5 cc.) + choline (0.5 g.)	–	±[b]	–	–	–

[a] 4 designates a reaction with definite particles visible; 3, 2, 1 indicates successively weaker reactions, each definitely not a negative reaction (–). [b] Indicates a doubtful reaction.

pletely satisfactory theoretical model. Enough of the important combinations have been considered, however, so that the equations give a satisfactory approximation of the theoretical system which would be formed by the species A, B, and H as postulated. With these considerations in mind, the following series of experiments was undertaken.

Experiments

The investigation of the effect of choline was undertaken as a result of the following speculation. Kahn antigen is lipoidal and some evidence suggests that a phospho-lipid component is important in similarly made antigens.[2] The effective component is presumably not cephalin, since results obtained with the cholesterol–cephalin test are not parallel with Kahn results.[3] The other well-known phospho-lipids contain choline, and this should be capable of forming fairly strong secondary bonds of the type needed to produce combination with an homologous antibody.

The first series of experiments deals with the effect of choline on standard Kahn precipitates, using positive sera obtained from the routine Kahn laboratory. Studies of three types were made: (1) the effect of relatively large amounts of choline for various antigen–antibody ratios, (2) the effect of smaller amounts of choline near the antigen-excess end of the zone of precipitation, and (3) the effect of small amounts of choline near the antibody-excess end of the zone.

To observe the first effect, 0.5 g. of solid choline chloride was dissolved in 0.5 cc. of serum, and tests were run by mixing 0.1 cc. of this solution with amounts of standard Kahn antigen suspension ranging from 0.005 to 0.080 cc. The tests were then completed and read according to standard Kahn procedure, as was the case in all subsequent experiments, unless otherwise indicated. The control consisted of serum diluted to an equal extent with 0.9% sodium chloride in water instead of choline, and otherwise treated in the same way. The result of this experiment is shown in Table I. It should be noted that no precipitate formed in the presence of this large amount of choline.

Two experiments were designed to investigate the influence of choline near the antigen-excess end of the zone. In the first of these serum dilutions were prepared using an approximately 10% solution of choline dissolved in 0.9% sodium chloride solution as diluent. As a basis of comparison, dilutions were also prepared using 0.9% sodium chloride alone; 0.15 cc. of these various dilutions were then mixed with 0.025 cc. antigen suspension as usual. It is clear from Table II that the choline prevents precipitation where it would otherwise occur. This same effect was also observed in sera G, H, J, and K of Table IV. These experiments were designed in connection with studies at the antibody-excess region.

TABLE II

EFFECT OF ADDITION OF CHOLINE TO SERUM BEFORE FORMATION OF PRECIPITATE. REGION OF ANTIGEN EXCESS

		Dilution factor				
	Diluent	5	10	20	30	40
Serum C	0.9% NaCl solution	4	4	–	–	–
	Choline in 0.9% NaCl soln.	4	–	–	–	–
Serum D	0.9% NaCl solution	4	4	±	–	–
	Choline in 0.9% NaCl soln.	–	–	–	–	–

In the second type of experiment at the antigen-excess part of the zone, serum dilutions were prepared with 0.9% sodium chloride solution, and the tests were carried to completion in the standard manner using 0.15 cc. of diluted serum and 0.025 cc. of antigen. Then choline (0.05 or 0.1 g.) was added in the form of a solution containing 0.5 g./cc., the tubes were incubated for five minutes at 37°, and the results were again read. Other additions of choline followed by incubation and readings were then made. Two of these experiments are shown in Table III. This and the preceding experiment indicate that choline has the same qualitative effect, whether it is added before or after the precipitate is formed.

TABLE III

EFFECT OF ADDITION OF CHOLINE AFTER FORMATION OF PRECIPITATE. REGION OF ANTIGEN EXCESS

	Serum E					Serum F				
Dilution factor	1	2	4	8	16	1	2	4	8	16
Initial reading	4	4	4	4	–	4	4	3	–	
Reading after first addition of choline	4	4	3	–	–	4	2	±	–	
Reading after second addition of choline	3	–	–	–	–	4	–	–	–	
Reading after third addition of choline	–					–	–	–	–	

(2) H. Eagle, "The Laboratory Diagnosis of Syphilis," Chapter III, C. V. Mosby Co., St. Louis, 1937.

(3) I. A. Mirsky, R. Brecht and L. D. Williams, *Science*, **99**, 20 (1944).

TABLE IV[a]

EFFECT OF ADDITION OF CHOLINE BEFORE FORMATION OF PRECIPITATE. REGION OF ANTIBODY EXCESS

	Antigen, cc.	0.005	0.010	0.020	0.040	0.080
	Serum, cc.	0.1	0.1	0.1	0.1	0.1
Serum G	Control	1	±	4	4	4
	Serum + choline	2	4	4	2	+
Serum H	Control	1	4	4	4	4
	Serum + choline	4	4	4	4	2
Serum I	Control	2	2	4		
	Serum + choline	3	4	4	3	–
Serum J[a]	Control	4	4	4	4	4
	Serum + choline	4	1	4	–	–
Serum K	Control	–	3	4	4	4
	Serum + choline	4	1	4	4	–
Serum L[b]	Control	–	±	4	4	4
	Serum + choline	4	4	4	4	4
Serum M[b]	Control	1	1	3	4	4
	Serum + choline	4	4	4	4	4

[a] Included only to show the effect of choline here observed at the antigen-excess end of the zone. [b] 0.15 serum was used here.

were carried out by first carrying through the tests without the addition of choline and reading the results. Then 0.05 g. of choline was added as a solution containing 0.5 g./cc., the tubes were reshaken for three minutes, and the results were again read. This was then followed by one or two more additions of choline, each followed by shaking and reading. It is clear from Table V that the addition of choline resulted in additional precipitation in the tubes containing the small amounts of antigen. A control treated in the same way, except that choline was not added, showed no significant change.

Besides these experiments with choline, similar but less extensive experiments were carried out with β-amino-ethanol. The amino-ethanol as obtained was in the form of the free base and consequently was neutralized before use with concentrated hydrochloric acid giving a solution approximately 40% with respect to the free base.

TABLE V

EFFECT OF ADDITION OF CHOLINE AFTER FORMATION OF PRECIPITATE. REGION OF ANTIBODY EXCESS

	Antigen, cc.	0.005	0.007	0.010	0.014	0.020	0.028	0.040	0.080
Serum N	Initial reading	±	1	4	4				
0.2 cc. per tube	Reading after first addition of choline	1	3	4	4				
	Reading after third addition of choline	4	4	4	4				
Serum O	Initial reading	–	±	2	4	4			
0.15 cc. per tube	Reading after first addition of choline	–	1	3	4	4			
	Reading after third addition of choline	2	3	4	4	4			
Serum P	Initial reading	±	±	1	4	4	4	4	
0.15 cc. per tube	Reading after first addition of choline	±	1	4	4	4	4	4	
	Reading after third addition of choline	1	2	4	4	4	4	4	
Serum Q	Initial reading	–		3		4		4	4
0.3 cc. per tube	Reading after first addition of choline	2		4					
	Reading after third addition of choline	2		4					
Serum R	Initial reading	±		3		4		4	4
0.3 cc. per tube	Reading after first addition of choline	±		4					
	Reading after third addition of choline	2		4					

The studies at the antibody-excess end of the zone were similarly carried out in two ways. First, to a small amount of each serum (often 0.5 cc.) was added 0.1 to 0.3 g. of solid choline. The increase in volume was noted, and controls were prepared by diluting another portion of the same serum to the indicated extent with 0.9% sodium chloride. Precipitation was carried out by adding the sera (0.1 or 0.15 cc., depending on the amount of serum available in each case) to various amounts of antigen suspension, as indicated in Table IV; 0.005 cc. of antigen was the smallest amount used, because it seemed to be the smallest amount that could be measured with reasonable accuracy with the available equipment. Sera for these experiments, and those to follow immediately, were especially chosen because they showed antibody-excess zoning in the routine examination. The results were entirely consistent; in the presence of choline, precipitation occurred with less antigen than in the absence of choline.

Further studies with small amounts of antigen It was found that it would inhibit the formation of precipitates near the antigen-excess end of the zone. The procedures used were entirely similar to those described for choline, 0.15 cc. of diluted serum and 0.025 cc. antigen being used, and the results are shown in Table VI. The procedures and results at the antibody-excess end of the zone were also entirely similar to those described in connection with choline. The results are shown in Tables VII and VIII.

TABLE VI[a]

EFFECT OF ADDITION OF β-AMINO ETHANOL TO SERUM BEFORE FORMATION OF PRECIPITATE. REGION OF ANTIGEN EXCESS

	Dilution factor				
Diluent	5	10	20	30	40
0.9% NaCl solution	4	4	3	–	–
Aminoethanol, (5%)	4	3	–	–	–
0.9% NaCl solution	4	4	4	1	–
Aminoethanol (15%)	4	1	–	–	–

[a] In this and the following tables the indicated groups correspond to different sera.

TABLE VII

EFFECT OF ADDITION OF β-AMINO ETHANOL BEFORE FORMATION OF PRECIPITATE. REGION OF ANTIBODY EXCESS

Antigen, cc.	0.005	0.010	0.020	0.040	0.080
Control	2	3	4	4	4
Serum (0.7 cc.) + amino ethanol (0.2 cc.)	4	4	4	4	4
Control	2	2	4	4	4
Serum (0.7 cc.) + amino ethanol (0.2 cc.)	4	4	4	4	4

TABLE VIII

EFFECT OF ADDITION OF β-AMINO ETHANOL AFTER FORMATION OF PRECIPITATE. REGION OF ANTIBODY EXCESS

Antigen, cc.	0.005	0.007	0.017	0.014	0.020	0.028	0.040
Initial reading	±	1	3	4	4		
Reading after first addition of amino ethanol (0.2 cc.)	2	3	4	4			
Reading after third addition of amino ethanol (0.2 cc.)	4	4	4	4			
Initial reading	—		3		4		4
Reading after first addition of amino ethanol (0.2 cc.)	±		4		4		4
Reading after third addition of amino ethanol (0.2 cc.)	2		4		4		4

Experiments at the zone ends were also undertaken using a fluid extracted from standard Kahn antigen. This fluid was prepared as follows. First, standard antigen suspension was made according to directions. This was next allowed to stand about fifteen minutes, and was then centrifuged for ten minutes at 2,000 r. p. m. The clear supernatant was then poured off and evaporated to about one-eighth its volume under reduced pressure in a 50–60° water-bath. This was to remove the ethyl alcohol from the solution, since it had been found that a serious interference results if it is allowed to remain. The effectiveness of this procedure was demonstrated with appropriate controls. The evaporated fluid was then diluted with distilled water so as to bring the final concentration of sodium chloride to either 0.9 or 2.5%, depending on the experiment for which the fluid was destined. The amount of water to be added was calculated on the basis of the assumption that the saline used in making the antigen suspension was the sole source of NaCl. The fluid thus diluted was ready for use, except that if any visible particles were present, they were removed either by centrifuging or by filtration through an asbestos mat.

The antigenic extract diluted to 0.9% sodium chloride was used as a serum diluent for studying its effect at the antigen-excess end of the zone, as described for choline. Controls consisted of serum similarly diluted with 0.9% sodium chloride in water. The results obtained were entirely consistent, and usually quite striking. Table IX shows that the extract had a definite inhibitory effect, as did choline.

TABLE IX

EFFECT OF ADDITION OF ANTIGENIC EXTRACT TO SERUM BEFORE FORMATION OF PRECIPITATE. REGION OF ANTIGEN EXCESS

Diluent	Dilution factor 5	10	20	30	40
0.9% NaCl solution	4	4	4	—	—
Antigenic extract	4	4	—	—	—
NaCl	4	4	4	4	2
Antigenic extract	4	—	—	—	—
NaCl	4	4	1	—	—
Antigenic extract	3	1	—	—	—
NaCl	4	4	±	—	—
Antigenic extract	3	±	—	—	—
NaCl	4	4	1	—	—
Antigenic extract	4	—	—	—	—
NaCl	4	4	±	—	—
Antigenic extract	±	—	—	—	—
NaCl	4	2	—	—	—
Antigenic extract	±	—	—	—	—
NaCl	4	4	—	—	—
Antigenic extract	4	±	—	—	—
NaCl	4	1	—	—	—
Antigenic extract—first extraction	1	—	—	—	—
Antigenic extract—second extn.	1	—	—	—	—
NaCl	4	4	±	—	—
Antigenic extract—first extraction	4	1	—	—	—
Antigenic extract—second extn.	4	±	—	—	—

The antigenic extract diluted to 2.5% sodium chloride was used to investigate the antibody-excess region of the zone, where unnecessary dilution is to be avoided. Here, equal amounts of this fluid and serum were mixed, and portions of this mixture were added to amounts of antigen ranging from 0.005 to 0.080 cc. For controls, serum was similarly mixed with 2.5% sodium chloride instead of the extract. As shown in Table X, again the results were impressively similar to those obtained with choline. Precipitation occurred with less antigen in the tubes containing serum diluted with the antigenic extract than in the corresponding ones containing serum diluted with 2.5% sodium chloride solution.

In no case was a precipitate observed when the antigenic extract was mixed with positive serum. This agrees with a similar lack of precipitate in sera due to the presence of either choline or amino-ethanol.

In one case, the residue from the original centrifuging of antigen suspension was resuspended in a mixture of ethanol and 0.9% sodium chloride. This mixture corresponded to the composition of the original supernatant. This suspension was recentrifuged, and the supernatant thus obtained was treated as was the original supernatant. The fluid thus formed was found to be effective in inhibiting precipitation near the antigen-excess end of the zone.

Discussion

In Figure 1 are illustrated the predicted effects

TABLE X

EFFECT OF ADDITION OF ANTIGENIC EXTRACT BEFORE FORMATION OF PRECIPITATE. REGION OF ANTIBODY EXCESS

Diluted serum, cc.	Antigen, cc.	0.005	0.010	0.020	0.040	0.080
0.3	Control	±	2	4	4	4
	Serum + antigenic extract	–	3	4	3	–
.3	Control	±	3	4	4	4
	Serum + antigenic extract	4	4	4	4	4
.2	Control	–	3	4	4	4
	Serum + antigenic extract	4	4	4	4	4
.2	Control	±	3	4	4	4
	Serum + antigenic extract	–	4	4	4	4
.4	Control	4	4	4	4	4
	Serum + antigenic extract	4	4	4	4	1
.2	Control	–	±	4	4	
	Serum + antigenic extract	4	4	4		
.1	Control	3	4	4	4	4
	Serum + antigenic extract	4	4	4	4	4
.15	Control	2	4	4	4	4
	Serum + antigenic extract	3	4	4	4	4

of a hapten H on the corresponding antigen-antibody precipitation. The results of the experiments with choline are in agreement with these predictions if choline is identified with the H substance of the theory. That is, choline was capable of completely preventing precipitation (Table I). Further, near the antigen-excess end of the zone it exhibited an inhibitory action (Tables II and III, points X, X′ of Fig. 1), and near the antibody-excess end of the zone it facilitated the formation of a precipitate (Tables IV and V, points Y, Y′ of Fig. 1). The theory is not only adequate to account for the experimental results, but a purely theoretical prediction was subsequently verified experimentally.

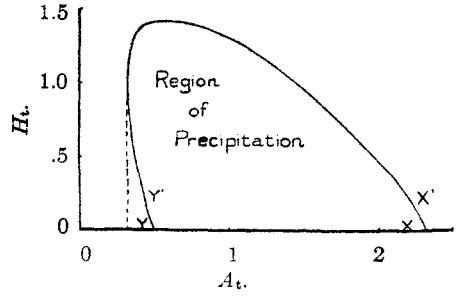

Fig. 1.

The theory was based on equilibrium considerations. Therefore, if the existence of the phenomena observed was shown to depend upon non-equilibrium effects, the theory could not be applied. However, the experiments indicate that equilibrium was at least approximated. The same result was obtained by approaching the final state from different directions in two cases (compare Table II with III, IV with V). Therefore, in this respect also, the theory may be satisfactorily applied to the observations.

The results with amino-ethanol and the extract of standard antigen may likewise be accounted for by the theory presented. An antibody capable of combining with choline might also be expected to combine with amino-ethanol, so that in view of the effects of choline, the results with amino-ethanol were not surprising.

The properties of the supernatant obtained from standard antigen suspension could be accounted for in two ways. The significant substance could be present in the liquid phase of the antigen suspension practically independently of its presence or absence in (or on) the solid phase. On the other hand, there could be a fairly rapidly attained equilibrium between the two phases. That the latter is the case is indicated by the fact that a second extraction of the solid phase yielded an effective extract.

The effect of an inhibitory substance at the zone ends should be correlated with the mechanism of the inhibition. Thus, for example, a substance reducing the effective amount of antigen would be expected to decrease the amount of precipitate at the antibody-excess end of the zone, but to increase it at the antigen-excess end.

The equations (1) to (8) may be interpreted as representing a system consisting of bivalent antigen, now represented by B, divalent antibody, represented by A, and monovalent antibody, represented by H. The curve of Figure 1 would then correspond to a relation between the two types of antibody. It is clear that the ratio of the amount of antibody at the two ends of the zone will now vary with changes in the ratio of monovalent and divalent antibodies in the serum. Large variations in zone width are a common observation in the serology of syphilis.

Summary

Theoretical considerations have led to the expectation that a monovalent hapten will have a different effect at the two ends of the zone of antigen-antibody precipitation. Near the antigen-excess end of the zone an inhibitory action is expected, while near the antibody-excess end of the zone the presence of the hapten should result in increased precipitation. Experiments have been carried out investigating the effect of each one of three substances, choline, β-amino-ethanol, and an antigenic extract, on the precipitation of syphilitic sera with standard Kahn antigen. Identification of these substances with the hapten of the theory results in complete qualitative accord between the experimental results and the theoretical predictions.

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