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Zone phenomena in the Kahn precipitation test for syphilis.

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The precipitation test for syphilis described by Kahn[1] is carried out with three tubes containing equal amounts of serum and diminishing amounts of antigen.

Three types of positive test are observed: In one, a strong precipitation occurs in the first tube, and progressively weaker reactions in the second and third; in the second type of positive test an equally strong precipitation occurs in all three tubes; in the third type, the precipitation is strongest in the third tube, and weaker in the second and first.

Zone phenomena in the Wassermann reaction were described in 1912 by Coca and L'Esperance[2] and more recently by Kahn and Johnson.[3] We have not observed zones similar to those described by these authors in which the strength of fixation increased as the antigen was diluted. We have, however, observed that very strongly positive sera when progressively diluted fixed with smaller amounts of antigen up to a certain maximum and in still higher dilution only with concentrated antigen.

The significance of such zone phenomena in precipitation and complement fixation reactions was carefully studied by Gay[4] and Dean.[5] Their findings have been confirmed by Parker[6] and by others who have studied the question. Dean pointed out that as a serum is diluted it is necessary to dilute the antigen correspondingly in order to obtain maximum fixation or precipitation and that consequently a serum which fixed or precipitated only with concentrated antigen, as a rule, had a higher antibody content than one which fixed with highly diluted antigen.

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[1] Kahn, R. L., *J. Am. Med. Assn.*, 1923, lxxxi, 88.

[2] Coca, A. F., and L'Esperance, E., *Ztschr. f. Immunitäts., Orig.*, 1912, xiv, 139.

[3] Kahn, R. L., and Johnson, S. R., *J. Infect. Dis.*, 1922, xxxi, 416.

[4] Gay, F. P., Univ. of Calif., Publications in Path, 1911, ii, 1.

[5] Dean, H. R., *Z. Immunitäts., Orig.*, 1912, xiii, 84.

[6] Parker, J. T., *J. Immunol.*, 1923, viii, 22.

TABLE I.
Fixation zone in Wassermann test.
Serum from untreated case of secondary syphilis.

Antigen 0.1 cc. dilution	Serum 0.05 cc.		Serum 0.02 cc.							No serum Control
	Undiluted	Undiluted	1—2	1—5	1—10	1—20	1—50	1—100	1—500	
1—10	++++	++++	++++	++++	++++	++++	++++	+++	0	0
1—20	++++	++++	++++	++++	++++	++++	+++	0	0	0
1—50	++++	++++	++++	++++	++++	++++	+++	0	0	0
1—100	+	++++	++++	++++	++++	++++	++	0	0	0
1—200	0	+++	++++	++++	++++	++++	+	0	0	0
1—500	0	0	++	++++	++++	+++	0	0	0	0
1—1000	0	0	0	0	+	+	0	0	0	
1—2000	0	0	0	0	0	0	0	0	0	
No antigen (control)	0	0	0	0	0	0	0	0	0	

TABLE II.
Precipitation zone in Kahn test.
Serum from untreated case of secondary syphilis.

Serum 0.3 cc.		Serum 0.15 cc.					
Antigen 0.3 cc. Dilution	Undiluted	Undiluted	1—2	1—5	1—10	1—20	1—50
1—1	++++	++++	++++	++++	++++	+++	0
1—2	++++	++++	++++	++++	++++	++++	0
1—5	+++	++++	++++	++++	++++	++++	++
1—10	+++	++++	++++	++++	++++	++++	+++
1—20	0	+++	++++	++++	++++	++++	+++
1—40	0	0	0	+	+++	+++	++

That the phenomena observed in the Kahn test follow this rule is shown by experiments such as that given in Table II, in which increasing dilutions of serum are tested with increasing dilutions of antigen. If one compares the first five results in column 1 with the corresponding results in column 4, it will be seen that by diluting the serum one can convert a reaction strongly positive in the first tube and negative in the last to one that is strongly positive throughout. Observing the results in the seventh column, it is seen that further increasing the dilution of serum converts the result to one negative in the first tube and strongly positive in the last. In other words, that the first type of reaction indicates a strongly reactive serum, the last type a weakly reactive serum.

The same result is arrived at if we take the strength of the cholesterin Wassermann test as an index of the strength of the serum.

TABLE III.
Correlation of Kahn and Wassermann reactions.
(Figures indicate number of sera giving each combination of results.)

Kahn Reaction			Wassermann Reaction (Cholesterol Antigen).				
1st tube	3rd tube	Negative	+	++	+++	++++	Average Wassermann val.
++++	0					11	4.0
+++	0					2	4.0
++	0					1	4.0
++++	+		1		1	12	3.7
++	+					1	4.0
++++	++				3	14	3.8
+++	++		1		1	2	3.0
++++	+++		1	2	3	14	3.5
++++	++++	3	4	1	26	87	3.5
+++	+++		1			2	3.0
++	++			1	1	3	3.4
+	+		1	1			1.5
+++	++++	2	4		4	8	2.8
++	++++	2	3		6	2	2.2
++	+++	1	2		2		1.6
+	++++	2	4		3		1.3
+	+++	1	2			1	1.5
+	++	1	2		1	1	1.8
0	++++	2	5		1		1.0
0	+++	4	7	2	1		1.0
0	++	17	8			1	0.46
0	+	21	4		1		0.27
0	0	667	7		1		0.015

In Table III are shown the Wassermann results obtained with 1,000 consecutive specimens, giving varying results with the Kahn test. The figures indicate the number of sera giving each combination of reaction. It will be seen that those in which a Kahn reaction of the first type (stronger in the first tube than in the last) was obtained, the average Wassermann value was 3+ to 4+; those giving the second type of Kahn test (equally strong in all three tubes) the average Wassermann value was 1.5+ to 3.5+; that in those giving the third type of Kahn test (stronger in the last tube than in the first) the mean Wassermann value was 0.3+ to 2.8+. To further study this point we titrated out the strength of the Wassermann in the number of sera giving a ++++ reaction and found that the very strong reactions were obtained only in those showing the first type of Kahn reaction.

These experiments are reported because they illustrate so well the principles laid down by Dean and because they are of some practical importance in interpreting the precipitation test. A true idea of the strength of the reaction cannot be obtained by averaging the values of the three tubes. The first type reaction described should be regarded as strongly positive, the third type as weakly positive, and the second as of intermediate strength.

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Tbe basal metabolism in vitamin B deficiency.

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The heat production in dogs during rest has been determined with the respiration calorimeter when these animals were fed with diets deficient in vitamin B. The purified food mixture fed to Dogs XXIV and XXV was complete in all respects with the exception of vitamins B and C. It was composed of casein, butter fat, lard, sucrose, salt mixture, and bone ash. Vitamin C

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