12 days after the injection of serum. The smallest amount of specific substance that caused a distinct but not maximal contraction, was 0.00000625 gm. added to a bath of 123 cc. so that the final concentration of the substance was 1:20,000,000. Desensitization of the uteri was demonstrated after the contraction due to one addition of 0.005 gm. of the substance to the bath.

When tested for sensitiveness to specific substances obtained from other strains of aerogenes there was no response, so that the results correspond to those obtained with the precipitin reaction.

As controls, the uteri from 6 normal guinea pigs were tested and not one gave a reaction following the addition of as much as 0.01 gm. of specific substance to the bath.

In vivo tests were made on 11 guinea pigs 24 hours after the intraperitoneal injection of from 1 to 4 cc. immune serum. Ten of these animals died showing the typical symptoms and signs of anaphylaxis. The M. L. D. of the specific substance for these sensitized animals was 0.000033 gm. The animal receiving 0.00002 gm., showed symptoms, but survived. Control tests on untreated animals show that 0.001 gm. failed to cause any reaction.

Attempts to sensitize guinea pigs with the specific substance alone were negative.

In this, which is a preliminary report, it is shown that in passively sensitized guinea pigs anaphylactic shock is produced by very high dilutions of a substance that is largely carbohydrate in nature. The presence of the small amount of nitrogen, presumably as an impurity, prevents us from concluding definitely that shock may be produced by carbohydrate alone. Further work with specific substances from other organisms is in progress.

3582

Optimal Proportions of Hapten and Immunserum in the Precipitation and Complement Fixation Reactions

JOSEPH TOMCSIK. (Introduced by Carl TenBroeck.)

From the Department of Pathology, Peking Union Medical College.

In a recent paper Dean and Webb[1] have shown that just as in the Ramon test there is an optimum zone in mixtures of protein and its antiserum where precipitation occurs more quickly, and that the indicator or first tube in which a precipitate appears contains neither

protein or antiserum in excess. They also find that after the reaction is completed the largest amount of precipitate is not found in this indicator tube. Dean's[2] previous work has shown that a mixture that gives the maximum precipitate is not the one that fixes the largest amount of complement.

TABLE I. *Precipitation.*

Antiserum dilution	Time of reading in minutes	Dilution of the yeast gum. Grade of the turbidity* and flocculation.												
		3,000	4,000	6,000	8,000	12,000	16,000	24,000	32,000	48,000	64,000	96,000	128,000	192,000	256,000
1:5	5	3	3	5	5	6	6	5	5	3	2	2	1	1	-
	12	4	4	6	7	±	7	6	5	3	2	2	2	2	1
	30	4	4	+	++	+	++	++	++	+	4	4	3	2	2
1:10	5	-	-	1	2	3	4	5	5	4	3	2	2	1	-
	30	-	1	2	3	4	5	+	6	5	5	4	2	2	2
	40	1	2	4	5	6	±	++	+	±	6	5	3	3	2
	90	4	6	7	7	±	+	++	++	+	+	±	±	5	5
1:20	5	-	-	-	-	-	1	2	3	3	3	2	2	2	1
	30	-	-	-	-	1	2	3	4	5	4	3	3	2	2
	60	-	-	1	2	4	5	6	6	7	6	6	4	3	3
	120	-	1	2	3	4	6	6	±	+	+	±	6	5	4

*The grade of the turbidity is given in arbitrary numbers from 1 to 7 and the amount of flocculation indicated by +.

Our object has been to study the quantitative relationship of antigen and antiserum in both the precipitin and complement fixation reactions in order to determine whether they are related. We have

TABLE II. *Complement Fixation.*

Antiserum dilution	Incubation time in minutes	Temperature	Units of complement	Dilution of the Yeast Gum. 24,000	32,000	48,000	64,000	96,000	128,000	192,000	256,000	384,000	512,000	768,000	1,024,000	1,536,000
1:20	1	room	20	++	+++	++++	++++	++++	+++	+	+	+	++	+	++	++
1:40	1	room	10													
1:80	1	room	2		+	+	+	+	+							
1:160	10	37°	2													
1:320	15	37°	2													

considered throughout the first tube giving precipitate or fixation so that our method differs from previous work where emphasis was placed on the maximum precipitate. Because of their great sensitivity we have used carbohydrate haptens rather than proteins as antigens. One of these haptens is a carbohydrate gum obtained from a pathogenic yeast, and the other the aerogenes specific substance described in the preceding paper. Both of these gave a precipitate with their respective immune serum when diluted 1:500,000 and complement fixation when diluted 1:64,000,000.

For the precipitation reaction the method of Dean and Webb was followed. Dilutions lower than 1:5 and higher than 1:40 could not be used, as in the former precipitate occurred at once in several tubes, and in the latter there was a slight opacity only.

It is difficult to restrict the fixation of complement to a few tubes, but by using varying amounts of complement and different incubation times and temperatures it is possible to determine for each dilution of serum the zone where maximum fixation takes place. Thus when the serum was diluted only 1:20 it was necessary to use 20 units of complement and to add the hemolytic system 1 minute after the complement.

In tables I and II are given the results obtained from tests made with the various dilutions of immune serum. It should be noted that for these tables we have selected the experiments where the narrowest zones of precipitation or complement fixation were obtained. It will be seen that when the dilution of antiserum is doubled, the dilution of hapten in the indicator tube is also doubled. Comparison of the two tables shows that approximately the same ratio of antiserum to antigen, 1:2400, in the indicator tube holds for both the precipitin and complement fixation tests in the various dilutions used.

Similar results showing that with serogenes specific substance the ratio of antiserum to antigen in the indicator tube is approximately the same in both precipitin and complement fixation reaction will be reported later in detail.

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[1] Dean, H. R., and Webb, R. A., *J. Path. and Bact.*, 1926, xxix, 473.

[2] Dean, H. R., *Zeit. f. Immun.*, 1912, xiii, 84.