QUANTITATIVE STUDIES ON ANAPHYLAXIS *IN VITRO*
I. PROPORTION OF ANTIGEN CONCENTRATION
TO TISSUE ANTIBODY CONCENTRATION IN
TOXIN-ANAPHYLAXIS OF DIPHTHERIA

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The relationship between the intensity of the serological reaction and quantities of antigen and antibody concerned has been studied on the serological reactions *in vitro*. As for the precipitin reaction, for example, the two-dimensional pattern of precipitating zone was reported by Boyd (1941) and confirmed by many investigators. Similar findings were also observed in complement fixation test. As anaphylaxis is one of the antigen-antibody reaction, it is conceivable that there would be some quantitative relation among the intensity of anaphylactic reaction, and the amounts of antigen and antibody concerned. In this connection, attempts were made to clarify the quantitative relation among them on toxin-anaphylaxis of diphtheria.

The quantitative studies on anaphylaxis have been carried out by many investigators. For example, Kabat and Landow (1942) studied on the relation between the amount of antibody used for sensitization and the amount of antigen required to cause anaphylactic shock to death. As the result of this experiment, it was found that the severest anaphylactic response occurred in the region of large antigen excess. However, it is clear that anaphylactic reaction is caused by antigen-antibody reaction in the tissues. In other words, the circulating antibody seems to take directly no part in anaphylaxis though sometimes it inhibits the occurrence of anaphylaxis (Ishizaka, 1953). Moreover, according to Beserga *et al.* (1953), it was confirmed that the concentration of circulating antibody has not necessarily any parallelism with the degree of sensitization. Therefore, in order to clarify the quantitative relation between the intensity of anaphylactic reaction and the antigen and antibody concerned, the influence of the circulating antibody on anaphylactic reaction must be excluded and the concentration of tissue antibody should be discussed.

On the other hand, it seems that one of the difficulties in quantitative studies on anaphylaxis is to exclude the individual difference of experimental

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animals. In order to eliminate the individual difference as well as the influence of circulating antibody, anaphylaxis *in vitro* with the aid of Schultz-Dale's method was applied in the previous report (Ishizaka *et al.*, 1954). Namely, the intensity of anaphylactic reaction was determined in terms of acetylcholine concentration and the concentration of antigen required to cause specific contraction comparable to the one caused by a certain concentration of acetylcholine was determined in each sensitized animal. As the result of this experiment, the correlation between the concentration of tissue antitoxin and the concentration of antigen required to cause a specific contraction in a certain degree was observed. However, the quantitative relation between them was not clear, because tissue antitoxin concentration was estimated from the disappearance rate of antitoxin from the circulation in the previous report.

In the present experiment, the concentration of tissue antitoxin was estimated exactly by using guinea pig's antitoxin labeled with I^{131} and the quantitative relation was studied in detail.

MATERIALS AND METHODS

Preparation of guinea pig's antitoxin labeled with I^{131} : For passive sensitization of guinea pigs, homologous antitoxin labeled with I^{131} was used. Pooled antitoxic serum obtained from hyperimmunized guinea pigs was fractionated according to Nichol *et al.* (1948) and crude γ -globulin fraction was obtained. Iodination of this fraction was performed according to the technic of Cohen (1951) with a slight modification. Namely, carrier free I^{131} (5 mc) was added to the approximate amount of solution of 0.09 N iodine in 0.1 N potassium iodide. After equilibration for 30 minutes in an ice-water bath, a chilled γ -globulin fraction of antitoxic serum which had been dialysed for 24 hours against borate buffer (pH 8.9) was added and the whole was kept in ice water for 1 hour. Then it was dialysed against repeated changes of phosphate buffer-saline in the cold for 72 hours. The radioactivity in the preparation not precipitable with trichloroacetic acid was 1%. The specific activity of the iodine solution added to the globulin solution and the activity per mg of protein nitrogen were determined. From these results, using 6.25 as the conversion factor from nitrogen to protein, the mean number of iodine atoms per molecule of protein of molecular weight 160,000 was calculated. Iodine combined was equivalent to an average of 0.27 atoms per globulin molecule. Antitoxin concentration of the preparation was determined by Jensen's intracutaneous rabbit method as well as precipitin test. It was 120 units/cc by both methods.

Determination of the degree of sensitization: Guinea pigs were sensitized by the intravenous injections of varying amounts of the preparation described above. Twenty-four hours later they were bled and their intestines were taken out. The degree of sensitization of each animal was determined by the method previously reported (Ishizaka *et al.*, 1954). Namely, the anaphylactic contraction of the intestinal strips against various amounts of purified toxoid (purity: 0.00055 mg protein-N/Lf) dropped into the bath was observed and its intensity was determined by the concentration of acetylcholine chloride required to cause an equivalent contraction. As shown in Fig. 1, the specific contractions of intestinal strips of a sensitized guinea pig caused by 0.11 Lf/cc, and 0.2 Lf/cc of purified toxoid in a Dale bath were equivalent to the contractions caused by acetylcholine at the concentration of 0.5×10^{-8} and 1.0×10^{-8} g/cc

respectively. With this method, the intensity of anaphylactic contraction can be expressed by the concentration of acetylcholine, and the individual difference in sensitivity to the effective substance of anaphylaxis can mostly be excluded. Moreover, by the application of this method, the concentration of antigen required to cause an anaphylactic contraction in a certain intensity (for example, anaphylactic contraction corresponding to the one caused by acetylcholine of 10^{-8} g/cc) can be determined in each animal. But, the contraction with acetylcholine appears generally very rapidly. On the other hand, when antigen is dropped into the bath, intestine begins to contract after a certain latent period and reaches the maximum gradually (Fig. 1). The velocity of

Fig. 1. Quantitative determination of anaphylactic contraction with acetylcholine.

A: acetylcholine chloride. T: diphtheria toxoid.

the contraction is different in each case. Therefore, it should be examined whether or not the concentration of acetylcholine can stand for the intensity of anaphylactic contraction. In this connection, the relation between the intensity of contraction and its velocity was studied as a basic experiment. As shown in Fig. 2, time in second, required for the contraction to reach the maximum from the beginning of contraction (b in Fig. 2) was determined in each case. The height of the curve (a in Fig. 2) was also determined in terms of acetylcholine concentration. In this case, a/b ($\tan \alpha$) represents the velocity of contraction. The correlation between $\log a$ and $\log a/b$ was calculated. As the result of calculation, correlation between them was significant at the risk of less than 1%, provided that the intestinal strip is normally sensitive to acetylcholine. In other words, the stronger is the intensity of anaphylactic contraction, the faster is the velocity of it. Therefore, the velocity of the contraction is dependent on its intensity expressed in terms of the concentration of acetylcholine and it has nothing to do with the concentration of antigen and/or tissue antibody.

As the result of this experiment, the experimental method was proved to be a suitable one for the determination of the degree of sensitization.

Determination of the concentration of tissue antitoxin: The concentration of tissue antitoxin in the intestine was estimated from its radioactivity. Following Pressman's method (1948), about 1 g of intestine to be measured for radioactivity

was macerated in distilled water, and to this was added 0.5 cc of 0.1 N potassium iodide solution as carrier, 1.5 cc of 1 N silver nitrate solution, 5 cc of concentrated nitric acid and 2.5 cc of 30% H_2O_2 . The flask was heated in a water bath at 80°C for 1 hour. An additional 2.5 cc of H_2O_2 was added and the heating was continued for another 1 hour. After cooling, the digest was centrifuged. The silver halide precipitate was washed with alcohol and ether and spread to a uniform thickness on aluminium holder and dried under a lamp. In order to avoid the significant self-absorption error, and

Fig. 2. Relationship between the intensity of anaphylactic contraction and the shape of the contracting curve.
 $\log a/b + \log 0.0415 = 1.00$ ($\log a + \log 0.848$)

geometric error, γ -globulin fraction of antitoxic serum used for sensitization as well as the serum of experimental animals, of which radioactivity was to be compared with that of intestine, were added to 0.5 cc of 0.1 N potassium iodide and 0.1 cc of 1 N silver nitrate and the whole mixture was spread on aluminium holder. The measurement of radioactivity was made with a Geiger Müller counter. Corrections were made for back ground, decay and when necessary, coincidence error.

Radioactivity of intestines and serums of sensitized animals was compared with that of I^{131} -labeled γ -globulin fraction used for sensitization. The concentration of tissue antitoxin and circulating antitoxin was calculated from radioactivity. By the way, circulating antitoxin concentration, determined by intracutaneous rabbit method, was in accordance with the one estimated from radioactivity.

In passively sensitized guinea pigs, concentration of tissue antitoxin was less than 0.4 units/g. In order to study on guinea pigs, in the case the concentration of tissue antitoxin is higher than this level, actively immunized guinea pigs were used. Several guinea pigs were immunized 3 times biweekly with alum-precipitated diphtheria toxoid, which was used to get antitoxin used for passive sensitization. Two weeks after the last immunization, they were bled and their intestines were taken out. For the determination of tissue antitoxin of these animals, tissue antitoxin was extracted with saline and was determined by intracutaneous rabbit method (Jensen, 1933). After the degree of sensitization was determined in each animal, other strips of intestine were homogenized by Waring blender and Potter type glass homogenizer. Then freezing and thawing was repeated 3 times and the homogenate was centrifuged at 10,000 g for 1 hour. The antitoxic titer of the supernatant was determined by intracutaneous rabbit method. As these guinea pigs were hyperimmunized ones, the avidity of their circulating antitoxin was high as reported by Jerne (1951). K_1 of these antitoxin was assumed to be 1.0, to calculate the concentration of tissue antitoxin. In this procedure, however, extraction of tissue antitoxin would be incomplete. Thus, some guinea pigs were passively immunized with I^{131} -labeled antitoxin, and 24 hours later, the concentration of tissue antitoxin in intestine was determined by intracutaneous rabbit method as well as by the measurement of radioactivity. As the result of the above, the concentration of tissue antitoxin estimated from radioactivity was found higher than that determined by the former method by about 20%. In this connection, the concentration of tissue antitoxin of actively immunized guinea pig was estimated to be 125% of that determined by intracutaneous rabbit method.

RESULTS

Anaphylaxis to diphtheria toxin was observed by Lawrence and Pappenheimer (1948) and experimentally demonstrated *in vitro* by the present authors (Ishizaka and Ishizaka, 1953). In this connection, it was confirmed that the contraction of intestine, sensitized with I^{131} -labeled antitoxin, against purified toxoid is caused by genuine toxin-antitoxin reaction. Namely, a guinea pig was sensitized with I^{131} -labeled antitoxin, and 24 hours later, its intestine was taken out. The doses of crude toxin and purified toxoid required to cause the same degree of anaphylactic contraction of the intestine were determined. As the result of this, the specific contractions caused by the same Lf of two antigens were equivalent to the control contraction of the intestine caused by acetylcholine in the same concentration. This finding is considered to give a basis to the opinion that these contractions of intestine were the anaphylactic reaction caused by the genuine diphtheria toxin (Ishizaka and Ishizaka, 1953).

1. *Relation between the concentration of tissue antitoxin and the concentration of toxoid required to cause an anaphylactic contraction in a certain intensity:* Guinea pigs were sensitized with varying doses of I^{131} -labeled antitoxin, and 24 hours later, they were bled and their intestines were taken out. By the application of the experimental procedure described above, the concentration of toxoid required for developing anaphylactic contraction comparable in the intensity to the one caused by acetylcholine in the concentration of 10^{-8} g/cc was determined for the intestine of each animal. After that, the intestines were

Table 1. Relationship between the tissue antitoxin and toxoid required to cause an anaphylactic reaction comparable to the one caused by acetylcholine in the concentration of 10^{-8} g/cc

No.	Body weight	Antitoxin injected	Circulating antitoxin	Tissue antitoxin	Antigen concentration required	Tissue-A Circulating-A
	g	u/100 g	u/cc	u/g	Lf/cc	%
1	430	27.9	1.67	0.083	0.15	4.98
2	425	28.2	1.89	0.0426	0.46	2.25
3	425	28.2	1.95	0.043	0.34	2.25
4	420	42.9	2.1	0.0597	0.23	2.84
5	340	21.2	1.01	0.0353	1.0	3.5
6	320	37.5	2.21	0.100	0.092	4.51
7	310	77.4	4.58	0.200	0.06	4.37
8	275	109.2	5.08	0.246	0.05	4.8
9	285	63.2	3.43	0.121	0.08	3.53
10	310	50.4	3.02	0.115	0.09	3.82
11	255	13.2	0.994	0.0294	1.1	2.9
12	260	23.0	1.31	0.0537	0.44	4.1
13	255	17.6	1.42	0.0343	0.99	2.42
14	260	34.6	1.70	0.0873	0.18	5.14
15	260	51.9	2.46	0.102	0.088	4.15
16	230	9.79	0.693	0.0258	2.2	3.75
17	245	45.9	2.36	0.0532	0.396	2.26
18	270	36.7	1.99	0.0642	0.22	3.22
19	285	31.6	1.71	0.0534	0.44	3.12
20	300	17.2	0.948	0.0400	0.44	4.22
21	305	9.6	0.47	0.0244	2.0	5.18
22	310	10.5	0.499	0.0242	1.5	4.85
23*	410	—	15.0	0.46	0.04	3.06
24*	380	—	20.0	0.57	0.046	2.85
25*	400	—	33.0	1.28	0.03	3.88
26	310	10.0	0.50	0.0244	11.0	4.88

* actively immunized

rinsed for one hour in Tyrode's solution and the radioactivity of the intestines were determined. The results obtained were shown in Table 1. In the next place, the concentration of toxoid required was plotted as ordinate against the concentration of tissue antitoxin as abscissa and the findings as shown in Fig. 3 were obtained. In this figure, both concentrations were represented in logarithmic scale. From this figure, it seems that the relation between them is not linear but hyperbolic. In this connection, it was tested statistically whether or not the data obtained are in accordance with the following equation:

$$(\log X + a) (\log Y + b) = c \quad (1)$$

Because the general equation of hyperbola is as follows:

$$2xy = k^2 \quad (2)$$

when its asymptotes fit axes. Three constants in equation (1) (a,b,c) were determined by the least square and equation (3) was obtained.

$$(\log X + 2.32) (\log Y + 2.48) = 2.058 \quad (3)$$

Equation (3) is the most reliable relation between the concentration of tissue antitoxin (X) and the concentration of toxoid (Y), required to cause anaphylactic contraction in a certain intensity (10^{-8} g/cc of acetylcholine), provided that the

Fig. 3. Proportion of antigen to antibody in anaphylaxis *in vitro* (1). Intensity of anaphylactic contraction was comparable to the one caused by acetylcholine in the concentration of 10 g/cc.

Curve: $(\log X + 2.32) (\log Y + 2.48) = 2.058$.

relation between concentration of antigen and antibody is hyperbolic. In the next place, $\log (\log X + 2.32)$ and $\log (\log Y + 2.48)$ were calculated in each animal and the correlation between them was tested. As the result of statistical test, it was found that the correlation between them was significant at the risk of less than 1% and that the relation is linear (Fig 4). The regression line was represented by the following equation:

$$\log (\log X + 2.32) + 0.995 \log (\log Y + 2.48) = 0.314 \quad (4)$$

Moreover, the regression coefficient was about -1.0 . In this connection, it was also tested whether or not the regression coefficient can be regarded as -1.0 , and this proved to be the case. In other words, it may safely be said that the relation between $\log X$ and $\log Y$ is hyperbolic and that each asymptote of the

Fig. 4. Proportion of antigen to antibody in anaphylaxis *in vitro* (2). $\log (\log X + 2.32) + 0.995 \log (\log Y + 2.48) = 0.314$
 X: $\log (\log X + 2.32)$ Y: $\log (\log Y + 2.48)$

hyperbola is parallel with the ordinate and abscissa respectively.

2. *Relation among the concentration of tissue antitoxin, the concentration of toxoid, and the intensity of anaphylactic contraction:* In order to generalize the findings described above, attempts were made to clarify the relation between antigen and antibody when the intensity of anaphylactic contraction was different. For this purpose, guinea pigs were sensitized with various amounts of antitoxin labeled with I^{131} , and 24 hours later, they were bled and their intestines were taken out. Then, various doses of toxoid were added to the bath, in which a strip of intestine had been suspended. The intensity of specific contraction was determined by acetylcholine, and the relation between the concentration of toxoid in the bath and the intensity of anaphylactic contraction was observed in each animal. At the same time, the concentration of tissue antitoxin was estimated from the radioactivity of the intestine. The results are shown in Fig. 5. In this figure, the abscissa represents the concentration of toxoid used and the ordinate represents the intensity of contraction expressed in terms of the concentration of acetylcholine required to cause an equivalent contraction. Numerals in this figure show the concentration of tissue antitoxin in each intestine. As the result of this experiment, it was confirmed that the higher the concentration of toxoid resulted in the production of so much stronger reaction. Namely, inhibition of the anaphylactic reaction due to antigen excess was never observed. The relation between the concentration of toxoid and the intensity of reaction was not linear. However, it seems that the higher the ratio antigen/antibody, the less the effect of the increase of antigen on the intensity of reaction.

The concentration of toxoid required to cause anaphylactic contraction comparable to the one caused by acetylcholine in the concentration of 1.4×10^{-8} g/cc

Fig. 5. Relation between concentration of toxoid and the intensity of anaphylactic contraction.

* Concentration of tissue antitoxin (units/g).

Table 2. Relation between the concentration of antigen and the intensity of anaphylactic reaction

No.	Antitoxin injected u/100g	Circulating antitoxin u/cc	Tissue antitoxin u/g	Antigen concentration† required to cause anaphylactic contraction		
				0.4×10^{-8}	1.0×10^{-8}	1.4×10^{-8}
1*	—	20.0	0.57	0.02	0.044	0.066
2*	—	33.0	1.28	0.015	0.03	0.044
3*	—	15.0	0.46	0.02	0.04	0.066
4	133.3	12.0	0.38	—	0.046	0.06
5	50.0	3.05	0.11	0.033	0.09	0.16
6	50.0	2.84	0.082	0.06	0.12	0.18
7	30.4	1.22	0.06	0.09	0.2	0.32
8	16.5	1.36	0.041	0.15	0.5	—
9	10.5	0.499	0.0242	0.2	1.0	—
10	18.6	1.08	0.033	0.3	1.0	—
11	38.2	1.52	0.0542	—	—	0.8
„	38.2	1.52	0.0542	—	—	1.0
12	17.2	0.948	0.039	—	0.54	0.88
„	17.2	0.948	0.039	—	0.54	1.1
13	38.0	2.3	0.1	—	0.1	0.15
14	21.3	1.05	0.05	0.1	0.3	—
15	23.7	1.26	0.051	—	0.285	0.4
16	30.5	1.52	0.052	0.1	0.27	0.4
17	72.3	4.30	0.15	—	0.07	0.15
18*	—	25.0	1.0	—	0.027	0.04
19*	—	25.0	0.9	0.02	0.033	0.04
20	35.6	1.74	0.055	0.1	0.24	—
21	125.3	9.25	0.37	—	0.046	0.056
22	20.5	0.98	0.05	0.1	0.3	—
23	41.6	2.28	0.112	0.04	0.088	—

* actively immunized

† Lf/cc

and/or 0.4×10^{-8} g/cc was estimated in each animal. The results are shown in Table 2. Then the relation between the concentration of tissue antitoxin and the concentration of toxoid in the bath, required to cause anaphylactic contraction comparable to the one caused by acetylcholine in the concentration of 1.4×10^{-8} g/cc or 0.4×10^{-8} g/cc was studied (Fig. 6). As the result of calculation, the most reliable equation expressing the relation was the following equation, when

Fig. 6. Relation among antigen, antibody and the intensity of anaphylactic contraction.

$$1.4: (\log X + 1.67) (\log Y + 1.62) = 0.515$$

$$1.0: (\log X + 2.32) (\log Y + 2.48) = 2.058$$

$$0.4: (\log X + 2.36) (\log Y + 2.35) = 1.39$$

the intensity of anaphylactic contraction is comparable to the one caused by acetylcholine in the concentration of 1.4×10^{-8} g/cc.

$$(\log X + 1.67) (\log Y + 1.62) = 0.52 \quad (5)$$

In the similar way, the following equation (6)

$$(\log X + 2.36) (\log Y + 2.35) = 1.39 \quad (6)$$

was obtained when the contraction is comparable to the one caused by acetylcholine in the concentration of 0.4×10^{-8} g/cc. As the result of chi-square test, it was confirmed that deviation of the data from these equations are not significant.

As the result of these experiments, the relation between the concentration

of tissue antitoxin and the concentration of antigen, required to cause anaphylactic contraction in a certain intensity would generally be represented by hyperbola of which asymptotes are parallel with the axes representing both concentrations.

Moreover, what is noteworthy is that apices of three hyperbola in Fig. 6 are near the equivalent point. In this connection, the following equation

$$(\log X + a) (\log Y + a) = c \quad (7)$$

was assumed and two constants (a and c) were determined by the least square. As the result of this, equation (8), (9), (10) were obtained.

$$0.4 \times 10^{-8}: (\log X + 2.35) (\log Y + 2.35) = 1.38 \quad (8)$$

$$1.0 \times 10^{-8}: (\log X + 2.20) (\log Y + 2.20) = 1.52 \quad (9)$$

$$1.4 \times 10^{-8}: (\log X + 1.63) (\log Y + 1.63) = 0.49 \quad (10)$$

It does not mean of course that these equations are not the most reliable ones, however, the deviations of experimental data from them were not significant. It is uncertain whether or not the apex of essential hyperbola representing the relation between antigen and antibody is on the equivalent point. However, it may safely be said that it is near the equivalent point.

DISCUSSION

Quantitative studies on anaphylaxis have been reported by some investigators. Especially, Kabat and Landow (1942) observed the relation between the doses of antibody used for sensitization and doses of antigen required to cause anaphylactic shock to death. In anaphylaxis, however, the individual differences of experimental animals are remarkable and the anaphylactic shock is too complicated for the elucidation of the quantitative relation between antigen and antibody concerned. In this connection, attempts were made by the present authors to eliminate the individual differences and to determine the intensity of anaphylactic reaction quantitatively. Namely, the intensity of anaphylactic contraction of sensitized intestine was determined by the concentration of acetylcholine, and the relation between the doses of diphtheria antitoxin used for sensitization and the doses of toxoid required to cause anaphylactic contraction in a certain intensity was studied (Ishizaka *et al.*, 1954). It is clear that the anaphylactic reaction is caused by antigen-antibody reaction in the tissues. Consequently, the concentration of tissue antibody should be determined in order to clarify the relation between antigen and antibody concerned in anaphylactic reaction. For this purpose, γ -globulin fraction of guinea pig's antitoxic serum was labeled with I^{131} , and the preparation was used for sensitization. Because, it was found by Knox *et al.* (1950) that if bovine serum albumin was lightly labeled with I^{131} , it could not be distinguished immunologically from the native protein. Moreover, according to Latta (1951), it was observed that radio-iodine labeled human albumin and bovine serum γ -globulin indicated slight change, if any, in their behaviour in the circulation in the experimental animal as compared with native

protein. Therefore, the use of I^{131} as a label appears very promising for the determination of tissue antibody. In fact, the disappearance rate of antitoxin from the circulation, estimated from radioactivity was in accordance with that of native antitoxin. In these considerations, concentration of tissue antitoxin as well as circulating antitoxin were estimated from their specific activity.

Thus, the relation between the concentration of tissue antitoxin and the concentration of antigen required to elicit an anaphylactic contraction of intestine in a certain intensity was studied. As the result of this experiment, it was observed that the relation is hyperbolic and that the apex of the hyperbola is near the equivalent point.

As for the quantitative relation between antigen and antibody, Kabat *et al.* (Kabat and Landow, 1942; Kabat and Boldt, 1944) reported that 0.03 mg N rabbit or guinea pig antiovalbumin or antipolysaccharide N was required to prepare the animal for uniformly fatal anaphylaxis when 1 mg (0.16 mg N) of ovalbumin or 0.1 mg of pneumococcal polysaccharide was injected intravenously 48 hours later. At the same time, they examined the effect of varying the amount of egg albumin used in shocking guinea pigs passively sensitized with known amounts of rabbit or guinea pig anti-egg albumin. As the result of this experiment, it was proved that the reaction is quite dependent on the amount of antigen used for eliciting shock. Such results can be explained by the present findings. Namely, as shown in Fig. 3, it is apparent that there is a threshold concentration of tissue antibody required to cause an anaphylactic reaction in a certain intensity. Moreover, when the concentration of tissue antitoxin is kept constant, the intensity of anaphylactic reaction is dependent on the concentration of antigen (Fig. 4).

According to the result of the experiment carried out by Kabat, Coffin and Smith (1947), an increase in the sensitizing dose of antibody from 0.03 to 0.15 mg N had little or no effect on the quantity of antigen necessary to kill one half of the animals by anaphylactic shock. This relation was observed in ovalbumin anti-ovalbumin system as well as S III anti-SIII system. This result can also be explained by the present findings, because, as shown in Fig. 5, the concentration of antigen required to cause a certain anaphylactic contraction (corresponding to 0.4×10^{-8} g/cc acetylcholine) is almost the same provided that the concentration of tissue antitoxin is more than about 0.5 units/g. Moreover, as the ratio of the tissue antitoxin concentration to the dosis of antitoxin used for sensitization is almost constant (2.5 to 5%), as shown in Table 1, the results obtained by Kabat *et al.* are explicable by the present findings.

Comparison of the relation between concentration of antigen and antibody required for anaphylactic reaction with those found in precipitin reaction shows very interesting findings. In precipitin reaction, it is said that the fundamental relation between antigen concentration and antibody concentration required to cause visible reaction within a certain period is parabolic, and that the apex of the parabola is on the equivalent point. In anaphylactic reaction, though the

fundamental relation is hyperbolic, apex of the hyperbola is near the equivalent point. Moreover, the concentrations of antigen and antibody required to cause a weak reaction are lower than the concentrations required for eliciting intensive reaction. These properties are similar to that observed in precipitin reaction. In precipitin reaction, however, the inhibition of visible reaction is observed in antigen excess (prozone). On the contrary, in the anaphylactic contraction of intestine, the more the concentration of antigen resulted in the production of so much stronger reaction even when the concentration of tissue antitoxin was low. For example, when the tissue antitoxin was 0.024 units/g, detectable contraction was not elicited by the equivalent concentration of antigen. The weak contraction was observed by 0.2 Lf/cc of toxoid and the specific reaction of standard strength (corresponding to 10^{-8} g/cc of acetylcholine) was elicited by 1.1 Lf/cc of toxoid. Moreover, when the concentration of toxoid was 11 Lf/cc, the anaphylactic contraction was equivalent to that caused by $1.0\sim 1.1 \times 10^{-8}$ g/cc of acetylcholine (Fig. 5). This concentration of antigen is about 458 times as much as its concentration equivalent to that of tissue antitoxin. Thus, the lacking of prozone would be one of the characteristic properties in anaphylactic reaction, though its reason remains unexplained.

SUMMARY

1. Anaphylactic contraction of guinea pig's intestine was studied quantitatively on diphtheria toxin-antitoxin system. Namely, the relationship between the concentration of tissue antitoxin and that of antigen required to cause an anaphylactic contraction in a certain intensity was studied.

2. When both concentrations were represented in terms of logarithmic scale, the relation between them was hyperbolic. Each asymptote of the hyperbola was parallel with the abscissa and ordinate, representing the concentration of tissue antitoxin and that of antigen respectively. Moreover, the apex of the hyperbola was in the neighbourhood of the equivalent point.

3. The quantitative relation of antigen and antibody in the anaphylaxis *in vitro* was discussed comparing with the pattern of precipitin reaction.

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