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STUDIES IN ANAPHYLAXIS[1]

XIV. ON THE RELATION BETWEEN PRECIPITIN AND SENSITIZIN

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In the following papers the term "Anaphylactic antibody" has been replaced by the word "Sensitizin." This has the advantage of brevity. The word is formed on the analogy of the words precipitin and agglutinin, and carries its own significance—namely that substance which confers sensitization. No distinctions are at present known between the substance responsible for active, and that for passive sensitization, so that both are covered by this term. Besredka some years ago suggested "Sensibilisin," but this is foreign to the spirit of the English tongue, being constructed on the root of the French verb "Sensibiliser" (to sensitize). "Anaphylactin," a term suggested by Anderson, had been used in a different sense by Gay and Southard, which seems to preclude its adoption.

Friedberger (5) was among the first to maintain the identity of precipitin and sensitizin, basing his belief on the fairly constant ratio between these two substances in various sera. Doerr and Russ (1) independently reached the same conclusion on the basis of a similar argument. This relationship, however, does not seem to be uniform, inasmuch as various observers have noted marked departures therefrom. Kraus and Novotny (6) after a careful experimental study of the conditions, categorically deny the identity of precipitin and sensitizin, and decline to accept the foregoing argument based on parallel ratios.

[1] The previous studies in this series were published in the Journal of Medical Research, 1913–1915.

The most recent attempt to determine the relations between precipitin and sensitizin was undertaken in 1914 by Lake, Osborne and Wells (7). They found that: "At the time the precipitin reaction appears, the passive anaphylactic condition usually can be induced in guinea pigs injected with this precipitating serum." They state their conclusions very cautiously as follows: "Inasmuch as the antisera which gave the precipitin test also caused passive anaphylaxis, it is possible that one and the same antibody is common to these two reactions." Doerr (2) summarizes the situation in the statement that the majority of workers tend to assume three different antibodies, in conformity with the three functions of precipitation, complement fixation, and sensitization.

Recent studies in the immunological aspect of human pneumonia seemed to me to lend renewed interest to this problem. Torrey and Weil (8) found that sensitizin was present during the disease, and generally absent after the crisis, whereas most observers have reported exactly the reverse of the occurrence of agglutinins, which generally appear only after the crisis. The question naturally arises as to the relationship of these two sorts of antibody, both to one another and to the evolution of the disease process. The following experiments seem to shed some light upon the latter question, and at the same time upon the long standing discussion concerning the identity of precipitin and sensitizin.

IDENTITY OF PRECIPITIN AND SENSITIZIN

There is one experiment, which does not seem to have been previously performed, that might bring the issue as regards the identity of precipitin and sensitizin nearer to a decision. If one could make use of the precipitate formed by the union of antigen and precipitin, to sensitize an animal passively, this result might be taken to indicate that precipitin may also act as sensitizin. After a considerable number of ineffectual attempts, it was found possible to accomplish this, as is evident from the following experiments.

Experiment 1. In this experiment the precipitin was supplied by the serum of rabbit 894, which had received repeated injections of horse serum. This animal was bled on June 11. On this date 0.1 cc. of the serum gave a heavy precipitate with 0.01 cc. of horse serum. Its sensitizing value is indicated by the following tests:

Guinea pig 852. June 11, 0.1 cc. 894 intraperitoneally.
June 12, 0.3 cc. horse serum intravenously. Moderate symptoms.

Guinea pig 853. June 11, 0.3 cc. 894 intraperitoneally.
June 12, 0.3 cc. horse serum intravenously. Immediate death.

On June 12 a series of tubes, each containing 1.5 cc. of Serum 894, was mixed with descending amounts of horse serum. The tubes were incubated for one hour, and then kept in the ice-box for forty-eight hours. The precipitation, which occurred almost immediately after mixing, is indicated in the following table:

TABLE I

Serum 894, 1.5 cc.

	HORSE SERUM	PRECIPITATE
	cc.	
(1)	0.3	+++
(2)	0.15	+++
(3)	0.05	++
(4)	0.02	+

The tubes were centrifuged and the supernatant fluid poured off. Five cubic centimeters of salt solution were added to the precipitate, and the tubes were again centrifuged. After pouring off the salt solution, the washed precipitate, which was considered to be practically free from all but traces of the original Serum 894, was employed in the following manner: To each tube was added 2 cc. of salt solution, and the precipitate thoroughly shaken up with it. On June 14 the mixture in each tube was injected intraperitoneally into a guinea pig of about

300 grams weight. Four days later each of these pigs received an intravenous injection of 0.5 cc. of horse serum. These details and the results are indicated in the following table:

TABLE II

Passive sensitization by washed precipitate

	WASHED PRECIPITATE OF SERUM 894, 1.5 CC. AND HORSE SERUM	GUINEA PIG	HORSE SERUM	SYMPTOMS
	cc.		*cc.*	
(1)	0.3	1	0.5	None
(2)	0.15	2	0.5	Very mild
(3)	0.05	3	0.5	Moderate
(4)	0.02	4	0.5	Death immediate

This table shows that a precipitate produced by the mixture of certain proportions of precipitinogen (horse serum) and precipitin (Serum 894) was competent to produce very effective passive sensitization of the guinea pig. Precipitates produced by a larger proportion of the horse serum were found to be decreasingly effective, while the first animal of the series, which receives the precipitate from the mixture in which the proportion of horse serum to precipitin was the largest, failed to give any evidence of sensitization.

In the preceding experiment 1.5 cc. of Serum 894 had been used, an amount which represented for this serum at least five sensitizing doses, as indicated by the preliminary sensitization tests previously detailed. In a second experiment the same serum was used in exactly the same manner, except that the amount was reduced to 0.5 cc. Horse serum was added in amounts proportional to those used before. The resulting precipitates were washed and injected into guinea pigs, which were tested four days later by the intravenous injection of horse serum. The results were as follows:

TABLE III

Passive sensitization by washed precipitate

	SERUM 894, 0.5 CC. AND HORSE SERUM	GUINEA PIG	HORSE SERUM	RESULT
	cc.		*cc.*	
(1)	0.1	1	0.3	No symptoms
(2)	0.05	2	0.3	No symptoms
(3)	0.018	3	0.3	No symptoms
(4)	0.007	4	0.3	Severe symptoms (convulsions)

These results indicate that not only the relative proportions of precipitin and precipitinogen, but also the absolute amount of precipitin, is a factor of importance in determining the capacity of a precipitate to induce passive sensitization.

The following table indicates a similar set of results, the minimal sensitizing dose of this rabbit serum being 0.5 cc. The processes in the series were exactly the same as those previously described.

TABLE IV

Passive sensitization by washed precipitate

IMMUNE RABBIT SERUM 190	HORSE SERUM	GUINEA PIG	HORSE SERUM	RESULTS
cc.	*cc.*		*cc.*	
2.0	0.2	1	0.3	No symptoms
2.0	0.02	2	0.3	Moderate symptoms
2.0	0.007	3	0.3	Very severe symptoms
1.0	0.02	4	0.3	Very mild symptoms
1.0	0.01	5	0.3	Very mild symptoms
0.8	0.001	6	0.4	Very severe symptoms
0.5	0.001	7	0.4	Moderate symptoms

In spite of the fact that these results seemed fairly conclusive, it was deemed advisable to carry the experiment one step further. Horse serum is a mixture of a number of antigenic substances, albumins and globulins, and this is a factor which might possibly in some unknown manner vitiate the results of the experiment. Crystalline egg albumen, therefore, was prepared by Finkuss'

method, being precipitated from solution three times. For this material I am indebted to Dr. A. F. Coca.

Experiment 2. Crystalline egg albumen was used to immunize rabbits 882 and 883. The serum of 882 sensitized guinea pigs passively in amounts of 0.1 cc., but not of 0.05 cc. The serum of 883 sensitized in amounts of 0.05 cc. Similarly, the precipitation reactions with 5 per cent of crystalline egg albumen showed that 883 was considerably richer than 882 in precipitins. These sera were employed to make precipitates with graded amounts of crystalline egg albumen, as in Experiment 1. The precipitates, after being washed twice, were injected into a series of guinea pigs, which were tested three days later by the intravenous injection of the egg albumen. The results are indicated in the following table:

TABLE V

Passive sensitization by washed precipitate

IMMUNE SERUM	CRYSTALLINE EGG ALBUMEN	PRECIPITATE	GUINEA PIG	EGG ALBUMEN	SYMPTOMS
cc.	*cc.*			*cc.*	
Serum 882:					
1	0.01	Slight	1	0.5	None
(10 sens. doses)					
1	0.001	++	2	0.5	Death at once
1	0.0001	Slight	3	0.5	None
Serum 883:					
1	0.1	None or very slight	4	0.3	Moderate
(20 sens. doses)					
1	0.01	+++	5	0.5	Death at once
1	0.001	++	6	0.4	Death at once
1	0.0,001	+	7	0.4	Mild
1	0.00,001	Slight	8	0.4	None

The fourth line in this table represents the so-called prozone in which excess of precipitinogen inhibits precipitation. Here the entire contents of the tube were injected.

A study of this table not only confirms, but very much strengthens the conclusions already reached. Precipitates pro-

duced by mixtures of precipitin and precipitinogen in proper proportions effectively sensitize guinea pigs passively towards the antigen. If a constant amount of immune serum be used, it is found that on each side of a zone of optimum proportions of antigen, lies a zone in which either an excess or a deficiency of antigen decreases the sensitizing value of the resulting precipitate. The explanation of this fact seems evident. An excess of antigen leads to incomplete precipitation of the antibody, part of which remains in solution, as in the prozone. A deficiency of antigen fails to carry down enough precipitate to sensitize. That this explanation is correct will be clear from the evidence submitted in a subsequent article.

The objection may arise that the sensitizing substance although carried down by the precipitate is not actually identical with the precipitin. The association might conceivably be purely mechanical, in other words the sensitizin might be carried down mechanically with the precipitate. In order to test this hypothesis, the following experiments were performed: One cubic centimeter of the serum of a rabbit immunized against horse serum (S. 894) was added to two sensitizing doses (0.1 cc.) of Serum 883 (rabbit versus egg albumen), and again to twenty sensitizing doses (1 cc.) of Serum 883. To each of these two mixtures 0.1 cc. of horse serum was added. Heavy precipitation occurred, owing to the reaction between 894 and horse serum. The supernatant fluid of the first tube was injected into a guinea pig, which responded fatally two days later to the intravenous injection of egg albumen. This shows that no appreciable amount of the sensitizing substances in Serum 883 had been mechanically carried down in the precipitate. The second tube was centrifuged, and the washed precipitate injected into a guinea pig. This guinea pig, when tested four days later by the intravenous injection of egg albumen, displayed no symptoms. This, again, shows that the washed precipitate failed to carry the sensitizing substances of Serum 883. The reverse experiment, in which Serum 894 was mixed with precipitating amounts of Serum 883 and egg albumen, had an identical outcome. Thus it seems clear that the mere act of precipitation does not mechanically carry down

the sensitizing substance. The conclusion seems unavoidable that the latter is identical with the precipitin. In any event, the burden of experimental proof certainly rests with those who might dispute this belief.

It has not invariably been found possible to contrive the proportions of precipitinogen and precipitin so as to produce passive sensitization with the precipitate. Thus Serum 775 (rabbit versus horse serum), which had a high precipitating titer, and which sensitized guinea pigs passively in amounts of 0.1 cc. was tested in the following combinations, without success.

TABLE VI

SERUM 775	HORSE SERUM	PRECIPITATE	WASHED PRECIPITATE INTO GUINEA PIG	HORSE SERUM INTRAVENOUSLY	SYMPTOMS
cc.	*cc.*			*cc.*	
0.5	0.5	+	1	0.5	None
0.5	0.01	++	2	0.5	None
0.5	0.002	+	3	0.5	None
1.0	0.1	+	4	0.5	None
1.0	0.01	++	5	0.5	None
1.0	0.001	+	6	0.5	None

The cause of failure with this particular serum is obscure, but was due, I believe, to the fineness of the precipitate, with consequent dissipation on washing.

The mechanism of sensitization by precipitates is discussed in a separate study.

SEPARATION OF THE PRECIPITATING FROM THE SENSITIZING FUNCTION

The demonstration of identity between precipitin and sensitizin does not, however, entirely dispose of the problem which relates to this substance. It might naturally be assumed, as a consequence of this identity, that wherever precipitin can be shown to exist, sensitizin will be present, and vice versa. As a matter of fact, Doerr (3) in his excellent summary of anaphylaxis in Kolle and Wassermann's Handbook, makes the statement

that these two modes of action of an immune serum are similarly affected by a variety of agents. For example, he specifies the fact that both precipitin and sensitizin resist the action of heat at 56°. Unfortunately for the theory, however, these relations are by no means as constant as might be inferred from Doerr's statement. Thus I have found that heating an immune serum may practically destroy the precipitin, while leaving the sensitizin quite intact. This fact is illustrated in the following experiment.

Experiment 3. Rabbit 775, highly immunized against horse serum, yielded a serum which passively sensitized a guinea pig of 300 grams in amounts of 0.1 cc. The precipitin tests showed that 0.1 cc. in 1 cc. of salt solution gave a distinct precipitate with 0.0001 of horse serum; with larger amounts of horse serum the precipitate was voluminous. One cubic centimeter of Serum 775, added to nine parts of 0.8 per cent salt solution, was heated for one-half hour at 70°. Precipitin tests with 3 cc. of this heated mixture failed to give any reaction with either 0.1, 0.01, of 0.001 of horse serum, though the tubes were observed several days. That the mere dilution could not be held responsible for this result is abundantly proven by Experiment 6 of this paper. On the day following inactivation, five guinea pigs were given subcutaneous inoculations of the heated mixture, three receiving 1 cc. each, and the remaining two, each 2 cc. The former group had an amount corresponding to one sensitizing dose, while the latter received two. Two days later all the animals were given intravenous injection of 0.5 cc. of horse serum. The first group showed symptoms of varying intensity, but none either had convulsions, or died. The two pigs in the second group, however, immediately died.

The same experiment was repeated several times, with identical results. Thus it seems that the serum contained sensitizin in only slightly diminished amount, or activity. In other words, heating at 70° had apparently destroyed the precipitin, but had only slightly affected the sensitizin. Apparently this fact demonstrates a striking difference between these two functions of the serum, and seems to argue for two separate substances, of which

one is thermolabile (precipitin), while the other is thermostable (sensitizin). It seems impossible at first sight to reconcile this observation with the fact that washed precipitate passively sensitizes guinea pigs.

An explanation, however, is quite possible, in keeping with the data of immunology previously ascertained and accepted. It is well known, from the work of Eisenberg and others, that heated precipitin, although it has lost the power of precipitating in the presence of antigen, still retains the capacity of uniting actively with the latter substance. Hence it is usually described as precipitoid or precipitinoid. In the terms of Ehrlich's hypothesis, it is said to have lost its ergophore or zymophore group, while it still retains its haptophore group. Now it is only necessary to assume that only the haptophore group need be present in order to sensitize a second animal passively. In offering this explanation, I wish simply to indicate the analogy of the new facts with those previously ascertained, without necessarily accepting the underlying theory.

Anaphylaxis, according to the theory which I have been led by the observations of numerous experiments to support, consists essentially in the interaction between anchored, or cellular, antibody, and freshly introduced antigen. For this reaction to supervene, it may reasonably be supposed that only the presence of the haptophore group of the antibody is necessary. In passive sensitization, the cells of the body appropriate the introduced antibody, and its characteristic haptophore group remains intact. With the injection of the specific antigen, the haptophore group of the cellular antibody seizes upon the latter, and as a result a cellular response, which constitutes the anaphylactic reaction, supervenes. Thus it becomes evident that the same immune substance may act either as precipitin or as sensitizin in its native state, whereas after heating it would lose the previous function, and retain only the latter. The effect of heat is, practically, to produce a new type of antibody which will sensitize while it does not precipitate. This fact suggests further reflections upon the identity of sensitizin and precipitin. It seems quite possible that the immunized animal may normally

produce antibodies of this same type, which have a haptophore, but no ergophore group. At present, one can only say that antibodies of this type do, indeed, occur; in fact this structure is supposed to be characteristic of the so called first order of antibodies of Ehrlich—the antitoxines. So far as is known, the latter have no sensitizing value, so that at present no sensitizing antibody without an ergophore group is known to exist normally.

There are certain other conditions under which it is possible to determine the presence of sensitizins, although precipitins can not be demonstrated. These observations are described in the following experiments.

Experiment 4. It is a well known fact that precipitating sera that give a voluminous precipitate with certain concentrations

TABLE VII

SERUM 894, 0.1 CC.	PRECIPITATE
Horse serum, 0.01 cc	+
Horse serum, 0.1 cc	Slight
Horse serum, 1.0 cc	−

of the antigen, may fail to give any precipitate with still higher concentrations of the antigen. This zone of absent precipitin reaction is sometimes described as the prozone. Its significance is not entirely clear. Eisenberg (4) believed that the precipitin is soluble in an excess of precipitinogen; others have maintained that the union of precipitinogen with precipitin produces a precipitate only when the latter factor is present in a certain excess. At all events, Eisenberg succeeded in demonstrating that the precipitin is firmly united to the precipitinogen in the fluid of the prozone. The limits of the prozone vary strikingly with various precipitinogens. In the case of horse serum, and of the serum of rabbits immunized thereto, the prozone may in some instances be manifest only when the antigen is present in excess of the antibody.

The addition of further antigen to the prozone mixture naturally produces no precipitation. If the prozone mixture in the above instance be injected into a guinea pig, passive sensitiza-

tion is not induced, owing to the fact that the great excess of antigen desensitizes the animal.

In the case of crystalline egg albumen, however, the relations as regards the prozone are entirely different, as shown by the following table:

TABLE VIII

SERUM 883, 1 CC.	PRECIPITATE
5 per cent crystalline egg albumen, 0.001 cc	++
5 per cent crystalline egg albumen, 0.01 cc	+
5 per cent crystalline egg albumen, 0.05 cc	−

Here the prozone is present in a combination in which the proportion of antigen to antibody is much lower than that shown in Table VII. Furthermore, experiment shows that 0.05 cc. of egg albumen does not completely desensitize a pig which has received 1 cc. of Serum 883. If, now, the prozone mixture here described be injected into a guinea pig, the latter becomes passively sensitized thereby, and after two days responds to an intravenous injection of antigen with marked and unmistakable anaphylactic symptoms, such as paralysis and dyspnoea, although death does not occur. Here, then, is another instance in which a fluid apparently contains sensitizin, but no precipitin.

Finally, one more illustration may be given of a condition in which a fluid may show no precipitin, although sensitizin is readily demonstrable. In this case the sodium carbonate extract of a precipitate is the fluid to be studied.

Experiment 5. In four separate tubes, 1 cc. of Serum 775 (rabbit versus horse serum) was added to 0.1 cc. of horse serum. On the following day, the resulting precipitates were washed twice in salt solution. To the washed precipitate in each of the tubes was added 1 cc. of 1 per cent sodium carbonate in water, the mixture being gently shaken. These tubes were incubated for one-half hour at 40°, according to the method of Gay and Chickering. The mixtures were then centrifuged and the supernatant fluid tested as follows: The supernatant fluid in two of the tubes were tested respectively against 0.01 and 0.001 of

horse serum, and failed to present any precipitation. The fluid from each of the other tubes was injected separately into two guinea pigs. Two days later these animals received an intravenous injection of 0.5 cc. of horse serum; both died in anaphylactic convulsions.

Here again it is clear that the so-called heat carbonate extract of the precipitate apparently possessed no precipitin, yet was amply supplied with sensitizin. The addition of fresh precipitin (Serum 775) to the heat carbonate extract produces a heavy precipitate. This demonstrates the presence of antigen (horse serum) in the extract. It might be assumed, therefore, that antigen and antibody are present in the extract in such proportions as to constitute a prozone. If this were the case, however, sensitization could not be induced, for the reason already given, namely that in the case of horse serum the prozone contains so much antigen as to preclude the possibility of passive sensitization. The constitution of the heat carbonate extract is not definitely known, but it seems clear that the antibody must be present in far larger proportion than in the prozone, while its precipitin, or zymophore, group, has been thrown out of action. The case is, therefore, analogous in some respects to the production of precipitoids by heat.

Thus by three different methods it is possible to produce a fluid in which the specific precipitins are apparently lost, while the sensitizins remain intact. In each of these instances the facts indicate that the haptophore group of the precipitin remains in part or in whole available, while the zymophore group is either neutralized or destroyed.

It remains to mention yet one more condition in which fluid containing precipitin shows marked diminution in its precipitating properties, while its sensitizing value remains intact. And this condition deserves special consideration, not only for the reason that it differs essentially in character from these already described, but also because it seems to offer a reasonable analogy with the conditions which may obtain during an infectious disease. Eisenberg, in his remarkable studies in precipitation, mentions the fact that exactly the same amounts of

precipitin and of precipitinogen which produce marked precipitation in the presence of relatively small amounts of a diluent, such as salt solution, fail completely to give a reaction when diluted with larger amounts. The following experiment illustrates the bearing of this fact on the question at issue.

Experiment 6.

Serum 775 (rabbit vs. horse serum)	0.1	cc.
Horse serum	0.01	cc.
0.8 per cent salt solution	1.0	cc.

Immediate flocculent precipitate.

Serum 775	0.1	cc.
Horse serum	0.01	cc.
0.8 per cent salt solution	10.0	cc.

No precipitation after incubation for one hour.

Complete precipitation after forty-eight hours in ice chest.

Although I am unable to confirm Eisenberg's belief that dilution inhibits precipitation inasmuch as it merely delays it, nevertheless within the ordinary limits of observation dilution would materially effect the results.

Serum 775, 0.1 cc. in 1 cc. salt solution, injected into a guinea pig. Three days later this animal received an intravenous injection of 0.5 cc. of horse serum. Immediate death.

The same dose of Serum 775, diluted with 10 cc. of salt solution, were injected into another guinea pig, of about the same size. The subsequent anaphylactic injection likewise produced immediate death.

In this experiment it is evident that mere dilution of the antibody serves markedly to delay precipitation of antigen. On the other hand, passive sensitization is in no wise affected by the dilution. The mechanism of the inhibition exerted by dilution upon precipitation is not entirely understood, but is believed to be physico-chemical in nature. Sensitization, however, depends simply on the absorption and appropriation of the antibody by cells.

The fact that precipitating antibody also possesses the func-

tion of passive sensitization, still leaves two questions open for discussion. In the first place, is all of the precipitating substance identical with sensitizin, or does a part of it exercise a precipitating, but not a sensitizing function? This is a question which can be answered only indirectly. Doerr states that in immune rabbit's serum the precipitating value is a reliable index of the sensitizing titer; in fact, he says the one can be predicted from the other. My own experience largely confirms this statement. On the theory of two different functions of precipitin, of which one alone exercises sensitizing functions, such parallelism seems almost impossible to explain. Moreover, there is no experimental ground for assuming such a condition. It may, therefore, be accepted that all precipitating antibody is also sensitizin. The second question is the reverse of the above: Is all sensitizing antibody capable of producing precipitation. Taken strictly, this question is to be answered in the negative, inasmuch as hemolysins may also act as sensitizins. There the lytic function appears to have replaced that of precipitation. But even on the broader basis it is probable that sensitizin may exist which entirely lacks an ergophore group. The fact that dissociation of precipitate in the test tube, as by sodium carbonate, leads to the production of an antibody which has these properties naturally leads to the expectation that dissociation within the body might lead to similar results. But at the present time our knowledge on the subject is so fragmentary that it is wiser to forego further speculation. At all events, the possibility of this factor should not be forgotten in considering the assertion that in some sera, especially in certain species, the sensitizin and the precipitin do not present parallel curves.

SUMMARY OF EXPERIMENTS

Identity of precipitin with sensitizin

1. Precipitates produced by a combination of horse serum and of the serum of a rabbit immunized against horse serum induce passive sensitization towards horse serum when injected intraperitoneally into a guinea pig.

2. The same results hold of precipitates produced by crystalline egg albumen and the serum of a rabbit immunized thereto.

3. In order to be effective in inducing passive sensitization, precipitates must result from mixtures of antigen and antibody in which the proportions between the two factors do not vary outside of certain fairly wide limits. Marked relative excess or deficiency of either factor produces a precipitate which fails to sensitize passively.

4. The sensitizing antibody is not merely adsorbed, or carried down mechanically with the precipitate. The precipitin is identical with the sensitizin.

Relations between precipitating and sensitizing functions of the antibody

5. If an immune serum be heated at 70° for half an hour, it loses its precipitating power, while its sensitizing value is but slightly impaired.

6. The admixture of antigen in excess inhibits precipitation, but affects sensitization in much slighter degree. The prozone fluid affords a striking illustration of this fact.

7. The heat carbonate extract sensitizes, but has no precipitating effect upon antigen.

8. High dilution of the antibody may markedly delay the precipitin reaction, while not affecting passive sensitization.

9. These data show that various agencies and conditions which inhibit precipitation may impair sensitization to only a moderate, or very slight degree.

THEORETICAL CONCLUSIONS

1. The precipitating substance of immune sera is competent to sensitize guinea pigs passively. In other words, precipitin is also sensitizin. It is conceivable, but improbable, that there may be a fraction of precipitin which lacks the sensitizing function.

2. Antibody may be deprived of its precipitating function by heat without suffering a very material diminution in its

sensitizing value. This observation corresponds with the previously known fact that "precipitoid," or heated precipitin, has retained its combining power with antigen, although it has lost its precipitating power. The precipitating, or ergophore group, is said to be thermolabile; the sensitizing, or haptophore group, to be thermostable.

3. Only the combining (or haptophore) group is essential to passive sensitization. Anaphylaxis therefore consists simply in the cellular reaction due to the fixation of antigen by cellular antibody. These new data therefore confirm and establish the theory of anaphylaxis supported in previous studies of this series.

4. The fact that the coexistence of antigen in the same fluid may inhibit the precipitating power of antibody, while only partially interfering with the sensitizing function, as in the prozone experiments, may explain the divergence in the literature between those who maintain that precipitin and sensitizin run parallel in immune sera and those who deny this relationship.

5. The phenomenon which has been described as dissociation of the precipitate, which probably occurs within the body, and which may be imitated by various laboratory procedures, such as extraction by sodium carbonate, sets free antibody in a form which sensitizes passively but fails to give the precipitin reaction. Such a factor, likewise, would upset the normal parallelism between sensitizin and precipitin.

6. The foregoing consideration may serve to explain the fact that the presence of antibodies may be demonstrated by means of passive sensitization in spite of the fact that the test tube reactions, such as agglutination and precipitation, prove ineffective. In infectious disease the co-existence of the antigen (the infectious agent or its product) in the blood might be expected to produce this result.

The terms ergophore and haptophore group in this paper are used merely as a convenient mode of expression, and without any theoretical implication.

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