THE NATURE OF ANAPHYLATOXIN.

Studies on Immunity. II.*

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It was shown by Friedberger (1) that the specific union between antigen, antibody, and complement is accompanied by the production of anaphylatoxin. If the mechanism of the Abderhalden reaction rests, as was suggested by my experiments (2), on the specific union of substratum and the specific fraction of immune serum, and if the presence of the complement is essential for the reaction to take place, there must appear during the reaction toxic products similar to those of Friedberger.

Experiments performed with this in view showed that the Abderhalden reaction is accompanied by the formation of highly active anaphylatoxin. These experiments were performed as follows.

The serum of three pregnant guinea pigs was mixed and placed in two centrifuge tubes, 6 c.c. in each, and about 3 gm. of boiled placenta tissue was added to

TABLE I.

Date.	Animal No.	Weight.	Dose.	Material injected.	Results.
Apr. 10...	160	250 gm.	3 c.c.	Supernatant fluid resulting from digestion of pregnant serum with placenta.	Death in 3 min.
Apr. 10...	162	255 gm.	2 c.c.		Death in 3 min.
Apr. 10...	163	255 gm.	1 c.c.		Death in 3 min.
Apr. 10...	165	260 gm.	0.5 c.c.		Grave symptoms. Recovery in 30 min.
Apr. 10...	167	250 gm.	2 c.c.	Supernatant fluid resulting from digestion of male serum with placenta (control).	No symptoms.
Apr. 10...	168	250 gm.	4 c.c.		No symptoms.
Apr. 10...	169	245 gm.	5 c.c.	Pregnant serum not having been digested with placenta (control).	Slight dyspnea.

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one of them. At the same time 6 c.c. of the serum of a male guinea pig was put in another centrifuge tube with 3 gm. of boiled human placenta tissue. All these tubes were placed in the incubator for sixteen hours, and at the end of this time the tubes were centrifuged, the serum was separated, and tested for toxicity by intravenous injection into normal male guinea pigs of about 250 gm. The results are shown in table I.

Parallel with this series, the same sera, both pregnant and male, were tested by the regular Abderhalden method with the result shown in table II.

TABLE II.

Pregnant guinea pig serum.		Male guinea pig serum.	
Serum 1.5 c.c. Placenta 0.5 gm.	Serum 1.5 c.c. No placenta	Serum 1.5 c.c. Placenta 0.5 gm.	Serum 1.5 c.c. No placenta
16 hrs. at 37° C. in dialyzing thimbles.			
Ninhydrin test +	Ninhydrin test −	Ninhydrin test −	Ninhydrin test −

As the above experiments suggest, the appearance of dialyzable substances on which the Abderhalden reaction depends, and the toxicity of the serum resulting from the interaction between the serum and the placenta tissue in the test-tube run parallel, and must, therefore, depend on some specific mechanism very similar or even identical in both cases. Since, however, it was suggested by the experiments reported previously (3) that the dialyzable split products appear in the Abderhalden test as a result of autodigestion of the serum, the results of the above experiment suggest that the appearance of anaphylatoxin in this experiment may be due to autodigestion of the serum. This conclusion would open the question of the mechanism of the formation of anaphylatoxin in general.

There exist at present two main theories explaining the origin of anaphylatoxin: one is a chemical theory developed by Friedberger (1910–1914); the other, first adopted by Doerr (4), explains the phenomenon on a physical basis. In his earliest experiments Friedberger (5) obtained from normal guinea pig serum which had been allowed to stand for some time with the washed specific precipitate (formed by rabbit serum immunized against sheep serum with the serum of the latter), very strong poisons which killed the guinea pigs instantly with symptoms of acute shock. He named the poisonous substance anaphylatoxin, and assumed that it arose from the digestion of the specific precipitate by the ferments of normal guinea pig serum. Later it was shown by Friedberger (6) and his pupils (7) that the anaphylatoxin may be derived also from different bacteria and other proteins by their incubation with normal sera without concurrence of specific antibody, and that the physiological action of the poisons thus obtained is similar

to that of the chemical poisons obtained previously by Vaughan and Wheeler (8). But Friedberger obtained them from various proteins by the action of proteolytic ferments of normal serum, whereas Vaughan and Wheeler obtained them from the same proteins by means of chemical agents.

According to the physical theory, the source of anaphylatoxin is not the protein of the antigen but the serum itself, which becomes toxic as a result of the removal of some substances from it, by means of physical adsorption. Ritz and Sachs (9), for instance, assume that the normal toxicity of the serum is masked by the presence of antagonistic substances which may be removed by adsorption. The recent experiments of Bordet (10) and those of Nathan (11) and Mutermilch (12) in which the substances causing the formation of anaphylatoxin by incubation with guinea pig serum, namely, agar, starch, and kaolin, are not of a protein nature, seem to give strong evidence in favor of the physical theory. The question, however, is not yet settled, as Friedberger (13) objected to the results obtained with agar and starch on the basis that these substances contain a small percentage of protein impurities and thus still may furnish the substratum for digestion.

If the interpretation given to the results obtained in the experiment above is correct, that is, if the toxicity of the end-product in the Abderhalden test is due to the autodigestion of the serum, the findings of Mutermilch can be easily explained, since it was shown in my previous publication (3) that the addition to the serum of kaolin causes the autodigestion of the serum.

However, since Friedberger failed to confirm the findings of Mutermilch, it was deemed necessary to establish first whether the digestion of the serum with kaolin is followed by the appearance of anaphylatoxin.

TABLE III.

Date.	Animal No.	Weight.	Dose.	Material injected.	Results.
Apr. 20...	311	255 gm.	1.5 c.c.	Serum treated with kaolin and incubated at 37° C.	Grave symptoms. Found dead next morning.
Apr. 20...	312	250 gm.	1 c.c.	Serum treated with kaolin and incubated at 37° C.	Grave symptoms. Recovery in 15 min.
Apr. 20...	313	255 gm.	4 c.c.	Untreated serum.	No symptoms.
Apr. 20...	314	250 gm.	3 c.c.	Serum treated with kaolin immediately after removal from ice.	No symptoms.

Freshly drawn guinea pig serum was placed in a centrifuge tube and immediately after the addition of an excess of sterile kaolin the tube was placed on ice, and, as soon as the kaolin settled out, a new portion of kaolin was added. Special care was taken that the kaolin should not be lumped, and that the particles of kaolin should be dispersed as uniformly as possible in the serum before they

were allowed to settle. After the serum had been treated in this manner three times, which took over nine hours, the contents of the tube were centrifuged, the serum was separated, and transferred to the incubator. After sixteen hours' incubation the serum was injected intravenously into normal guinea pigs weighing about 250 gm., and was found to be toxic, as shown in table III.

Since kaolin is not soluble, it is evident that the toxicity of the supernatant fluid is due to changes in the serum. The possible objection that particles of kaolin may be suspended in this serum, and thus cause harm mechanically or chemically, is obviated by the fact that, as the experiment shows, the same serum, although toxic after incubation in the thermostat, is not toxic immediately after removal from kaolin.

As to the nature of the changes which may have taken place in the serum, my experiments show that the contact with kaolin deprives the serum of its antitrypsin and that subsequent incubation at 37° C. is followed by the autodigestion of the serum.

DOES PLACENTA FURNISH TOXIC PRODUCTS IN THE ABDERHALDEN REACTION?

In view of the results discussed above, the suggestion that the anaphylatoxin formed during the Abderhalden test originated from the serum seems to be strengthened. However, since in one instance the serum is digested with kaolin, which by itself cannot be the source of digestible material, whereas in the other instance serum is combined with a placenta tissue, and especially since it is asserted by Abderhalden that in these conditions of the experiment placenta is digested, it was deemed necessary to arrange the experiment so as to avoid even the possibility of the digestion of placenta before it would be possible to determine the source of the toxic material in this case. This was attempted in the following experiment (table IV).

12 c.c. of pregnant guinea pig serum were placed on ice in a tube with about 5 gm. of boiled human placenta tissue. Sixteen hours later the contents of the tube were centrifuged and the supernatant fluid[1] was separated from the sediment. 8 c.c. of this fluid were transferred into another tube and placed in the incubator (37° C.) for sixteen hours. At the end of this time the contents of

[1] Serum treated in this way, as was shown before, is deprived of its specific constituents as well as of the natural antitryptic inhibition, and if placed at this stage in the incubator it undergoes autodigestion.

the tube were tested for toxicity by the intravenous inoculation in normal male guinea pigs. The remaining 3 c.c. of supernatant fluid collected after centrifugation were tested for toxicity immediately after centrifuging.

TABLE IV.

Date.	Animal No.	Weight.	Dose.	Material injected.	Results.
Apr. 13...	200	250 gm.	3 c.c.	Supernatant fluid before incubation.	No symptoms.
Apr. 14...	202	253 gm.	3 c.c.	Supernatant fluid after incubation at 37 ° C. for 16 hrs.	Death in 2 min.
Apr. 14...	203	255 gm.	2 c.c.		Death in 2 min.
Apr. 14...	204	255 gm.	1 c.c.		Death in 2 min.
Apr. 14...	205	260 gm.	0.5 c.c.		Death in 5 min.
Apr. 14...	206	250 gm.	0.25 c.c.		Acute symptoms. Recovery in 15 min.
Apr. 14...	207	250 gm.	0.5 c.c.		Death in 3 min

As in the preceding experiment, here again the serum is not toxic immediately after the separation from placenta, but it becomes toxic in the absence of placenta if allowed to remain in the incubator, thus suggesting that the serum and not the placenta is the source of poison.

However, this conclusion is open to the criticism that the possibility of the serum containing minute particles of placenta in suspension after centrifugation is not definitely excluded. Such particles of placenta, although not resulting in toxicity of the serum immediately after removal from ice, may be digested later when the serum is transferred to the incubator, and thus contribute to the toxicity of the fluid. In order to show that such particles of placenta could not become the source of anaphylatoxin, the following experiment was undertaken (table V).

Pregnant human serum was placed in two centrifuge tubes, 5 c.c. in each, with about 2 gm. of human placenta protein. In two other centrifuge tubes pregnant guinea pig serum, 5 c.c. in each, was placed, again with 2 gm. of human placenta protein. Another exactly similar series of four tubes was set up with guinea pig instead of human placenta. All the tubes were placed for sixteen hours in the ice box, at the end of which time they were centrifuged and the serum was separated. One tube of each set was transferred into the thermostat for sixteen hours, to be injected subsequently into guinea pigs; the other tube of each set was tested immediately after the separation from the substratum.

If the toxic substances in the experiments above originated from

TABLE V.

Toxicity of Sera before Incubation.

Date.	Animal No.	Weight.	Dose.	Material injected.	Results.
Apr. 17	211	265 gm.	2 c.c.	Human serum + human placenta	Death in 10 min.
Apr. 17	212	260 gm.	1.5 c.c.	Human serum + human placenta	Mild symptoms.
Apr. 17	214	250 gm.	1.5 c.c.	Human serum + guinea pig placenta	Mild symptoms.
Apr. 17	215	250 gm.	3 c.c.	Guinea pig serum + human placenta	No symptoms.
Apr. 17	216	248 gm.	4 c.c.	Guinea pig serum + guinea pig placenta	No symptoms.
Toxicity of the Same Sera after Incubation at 37° C. for Sixteen Hours.					
Apr. 18	219	255 gm.	2 c.c.	Human serum + human placenta	No symptoms.
Apr. 18	220	250 gm.	3 c.c.	Human serum + human placenta	No symptoms.
Apr. 18	221	258 gm.	4 c.c.	Human serum + guinea pig placenta	No symptoms.
Apr. 18	226	255 gm.	1 c.c.	Guinea pig serum + human placenta	Death in 5 min.
Apr. 18	227	260 gm.	0.5 c.c.	Guinea pig serum + human placenta	Death in 5 min.
Apr. 18	228	260 gm.	0.25 c.c.	Guinea pig serum + human placenta	Coughing; recovery in 15 min.
Apr. 18	229	258 gm.	0.5 c.c.	Guinea pig serum + guinea pig placenta	Death in 3 min.
Apr. 18	231	250 gm.	0.25 c.c.	Guinea pig serum + guinea pig placenta	Mild symptoms for 10 min.; recovery.
Apr. 18	233	250 gm.	0.5 c.c.	Guinea pig serum + guinea pig placenta	Death in 5 min.

the minute particles of tissue remaining in suspension after centrifuging, then the fluid injected into guinea pigs 219 or 220 and that injected into 226 or 227 should be equally toxic, since in both cases the sera were digested with the same tissue. The same should be true about the toxicity of the portions of human and guinea pig sera, respectively, both of which were digested with guinea pig placenta. As can be seen from the results, however, whereas the toxicity of guinea pig serum increased during the incubation more than 800 per cent., the human serum, on the contrary, lost its natural toxicity for guinea pigs. The results of this experiment seem to show conclusively that the toxic substances in the Abderhalden test originate from the serum.

DIGESTED SERUM IS TOXIC ONLY FOR HOMOLOGOUS ANIMALS.

Although the results of the preceding experiment show that the toxicity of the end-products of the Abderhalden reaction originates

from the serum, the fact that the pregnant human serum, having been treated in a way absolutely similar to that of a pregnant guinea pig, failed to develop toxic properties suggests further study. There seem to be two possible explanations of this difference. Either the split products of human serum are in general less toxic than those of guinea pig serum, or the products of digestion of any serum are toxic to homologous animals only. In order to determine which is the case, the following experiments were undertaken.

On Apr. 21 5 c.c. of the serum of a pregnant rabbit and guinea pig, respectively, were put in test-tubes with 2 gm. of boiled human placenta tissue in each and left on ice over night. A similar set of two tubes was made up with human placenta and male instead of pregnant serum of a rabbit and a guinea pig. Early the next morning the contents of all four tubes were centrifuged, and after separation from the sediment the sera were transferred to the thermostat for sixteen hours. At the end of this time the contents of the tubes were injected intravenously into male guinea pigs and rabbits with the results indicated in table VI.

TABLE VI.

Date	Animal.	Animal No.	Weight.	Dose.	Material injected.	Results.
Apr. 22	Rabbit	101	1,075 gm.	2 c.c.	Pregnant rabbit serum	Acute symptoms. Found dead next morning.
Apr. 22	Rabbit	102	1,090 gm.	1 c.c.	Pregnant rabbit serum	Grave symptoms. Recovered.
Apr. 22	Rabbit	103	1,015 gm.	5 c.c.	Male rabbit serum	No symptoms.
Apr. 22	Rabbit	104	1,025 gm.	3 c.c.	Pregnant guinea pig serum	No symptoms.
Apr. 22	Rabbit	105	1,025 gm.	3 c.c.	Male guinea pig serum	Slight symptoms; recovered in 15 min.
Apr. 22	Guinea pig	306	255 gm.	1.5 c.c.	Pregnant rabbit serum	Restless; recovered in 5 min.
Apr. 22	Guinea pig	307	250 gm.	1.5 c.c.	Male rabbit serum	Coughing; recovered in 30 min.
Apr. 22	Guinea pig	308	255 gm.	0.5 c.c.	Pregnant guinea pig serum	Dead in 2 min.
Apr. 22	Guinea pig	309	245 gm.	4 c.c.	Male guinea pig serum	No symptoms.

It would seem, therefore, that the products of autodigestion of human serum must be toxic for human beings if they are toxic at all. In order to determine if this was the case, serum of a pregnant woman was treated in a way exactly similar to that just described for the serum of a rabbit or guinea pig. After separation from the placenta and subsequent incubation at 37° C., it was injected at in-

tervals during the process of autodigestion in the dose of 0.05 c.c. into the skin of several members of the laboratory staff. Parallel injections were made also with 0.05 c.c. of male serum treated in a similar manner. Whereas the injections of male serum did not cause any symptoms, the pregnant serum showed unmistakable signs of toxicity. Moreover, this toxicity seemed to be increasing for a certain time with the progress of digestion, since the injection made six or twelve hours after the beginning of autodigestion did not cause any symptoms. The injection of the material digested eighteen, twenty-four, and thirty hours was followed by a strong local reaction which resulted in the first two cases in a distinct reddened area with marked sensitiveness, and in the last case in the production of a sterile pustule, which appeared about ten hours after injection and remained for over twenty-four hours (14). The reactions at the site of the inoculation with the same serum later in the process of autodigestion showed marked diminution in toxicity of the serum, and after forty-eight hours the serum lost all its toxicity.[2]

TOXICITY IS CHARACTERISTIC ONLY OF A CERTAIN STAGE OF THE AUTODIGESTION OF THE SERUM.

In the experiments reported above it was assumed on the basis of previous results that toxicity of the serum, previously treated with kaolin or placenta protein, respectively, developed during its subsequent incubation at 37° C. is due to the process of autodigestion. If this is true, the loss of toxicity of such a serum, if incubation is prolonged, may be due to the further cleavage of the split products of the serum beyond the toxic stage. That both these assumptions are correct may be seen from the following experiments (table VII).

6 c.c. of pregnant guinea pig serum were placed in four test-tubes, 1.5 c.c. in each, and left on ice for sixteen hours in contact with 0.5 gm. of boiled human

[2] Similar findings were reported by Vaughan, Cumming, and Wright (15). According to these authors the serum or extracts of organs from actively anaphylactic guinea pigs after half an hour's contact with antigen furnished (intracardial injection) poisons which were acutely fatal, while sera or extracts of organs of normal animals did not. These authors report that after longer contact these poisons were destroyed.

placenta tissue in each tube. At the end of this time the contents of all tubes were thoroughly centrifuged, the serum was separated and transferred to four tubes containing 4.5 c.c. of physiological salt solution, 4.5 c.c. of normal guinea pig serum, 4.5 c.c. of solution of serum albumin, and 4.5 c.c. of a solution of serum globulin, respectively. All the tubes were placed in the incubator and at the end of sixteen hours the contents were injected intravenously into normal guinea pigs.

TABLE VII.

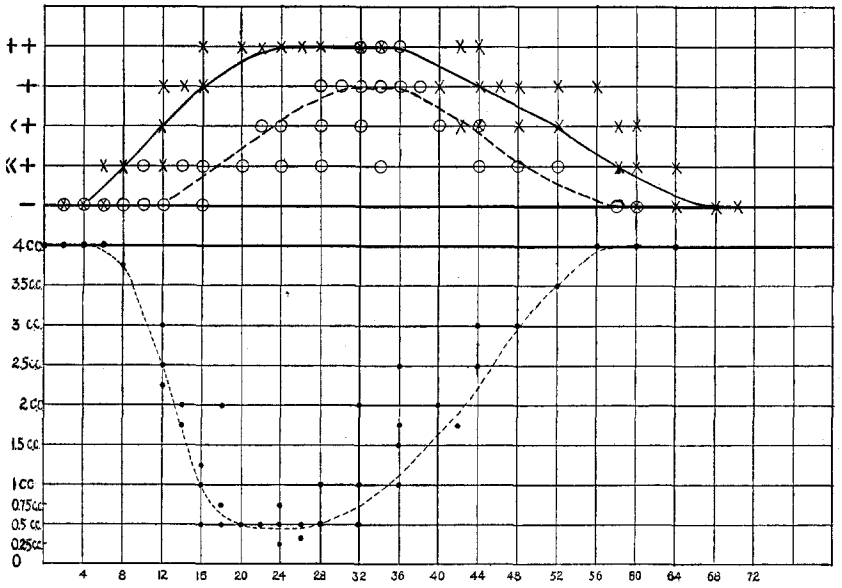
Date.	Animal No.	Weight.	Dose.	Material injected.	Results.
May 8	320	240 gm.	1 c.c.	Treated serum + salt solution	No symptoms.
May 8	321	250 gm.	1.5 c.c.	Treated serum + salt solution	Grave symptoms. Recovery.
May 8	322	250 gm.	2 c.c.	Treated serum + salt solution	Death in 2 min.
May 8	323	240 gm.	4 c.c.	Treated serum + normal serum	Very mild symptoms.
May 8	324	250 gm.	4 c.c.	Treated serum + serum albumin	Mild symptoms.
May 8	326	253 gm.	4 c.c.	Treated serum + serum globulin	Death in 2 min.
May 8	327	255 gm.	1.5 c.c.	Treated serum + serum globulin	Grave symptoms. Found dead next morning.

It is evident that the excess of normal serum or serum albumin, which, as I showed (16), stops the autodigestion of serum, as tested by the appearance of dialyzable ninhydrin reacting substances, prevents also the formation of toxic products in the serum. Serum globulin, which was shown to promote the autodigestion of serum, does not stop the formation of anaphylatoxin. That such toxic sera, during prolonged incubation, lose their toxicity due to further digestion of toxic compounds, can be seen from the following experiment.

On May 2 at 4 P.M. five pregnant guinea pigs were bled, and 40 c.c. of the serum were placed on ice in a tube with 20 gm. of boiled human placenta. At 8 A.M. on May 3, after sixteen hours, the serum was separated by centrifuging and transferred to the thermostat. In the progress of incubation samples of the serum were taken at intervals and tested simultaneously for toxicity upon normal guinea pigs as well as for dialyzable products of digestion. The latter test was done by transferring 1.5 c.c. of the serum from the incubator into a dialyzing thimble, as for the Abderhalden test (but without substratum), and placing the container with the thimble on ice. Under these circumstances, as I have shown, no further digestion takes place, and if there are any dialyzable products in the serum, they are not prevented from diffusing into the outer liquid in the container. Dialysis is allowed to proceed for twelve hours and at the end of this time the ninhydrin as well as biuret tests are made. The results of the experiment are shown in table VIII.

TABLE VIII.

Date.	Duration of incubation.	Weight.	Dose.	Results.	Biuret test.	Ninhydrin test.
May 3, 3 P.M.	6 hrs.	250 gm.	0.5 c.c.	No symptoms	−	−
May 3, 9 P.M.	12 hrs.	250 gm.	0.5 c.c.	Death in 10 min.	−	< +
May 4, 9 A.M.	24 hrs.	245 gm.	0.5 c.c.	Death in 5 min.	< +	+ +
May 4, 9 P.M.	36 hrs.	240 gm.	0.5 c.c.	Grave symptoms Recovery	≪ +	+ +
May 4, 9 P.M.	36 hrs.	245 gm.	1 c.c.	Death in 10 min.	≪ +	+
May 5, 9 A.M.	48 hrs.	250 gm.	1 c.c.	No symptoms	−	≪ +
May 5, 9 A.M.	48 hrs.	250 gm.	2 c.c.	Mild symptoms	−	≪ +
May 5, 9 A.M.	60 hrs.	255 gm.	2 c.c.	No symptoms	−	−
May 6, 9 A.M.	72 hrs.	253 gm.	2 c.c.	No symptoms	−	−



TEXT-FIG. 1. The average changes of the serum, recorded in several successive experiments. The numbers placed on abscissæ represent the intervals of time at which the examinations were made. The ordinates show the intensity of the respective reactions. The lowest dotted curve represents the toxicity developed in the serum during incubation, the ordinates expressing the volume of the minimal toxic dose, which is inversely proportional to the toxicity of serum. The two upper curves represent the intensity of the biuret (dotted line) and ninhydrin (solid line) tests and are expressed in arbitrary terms of negative, very weakly positive, weakly positive, positive, and strongly positive reactions.

The results, however, are not absolutely constant, since the rate of digestion may be influenced by the amount of antibody as well as by the antitryptic index, both of which vary in individual cases, and if both happen to exert their influence in the same direction the autodigestion may be markedly delayed or accelerated, as the case may be.

Comparison of these curves suggests definitely that the toxicity of the serum is in direct relation to its digestion, and that, if this digestion is allowed to proceed far enough, the cleavage products are broken down beyond the stage at which they possess the molecular configuration responsible both for the toxicity as well as for the typical color reactions (text-figure 1).

DISCUSSION.

Assuming that the anaphylactic syndrome, as it occurs in the body of a sensitized animal upon introduction of antigen, is due to the toxicity of the products of specific parenteral digestion of antigen, Friedberger attempted to produce the same substances *in vitro* by adding fresh complement to the antigen-antibody combination. The poisons obtained in this way, when injected in normal animals, were able to produce typical anaphylactic phenomena. Assuming that the substance obtained *in vitro* was identical with that formed *in vivo* in anaphylaxis, he called this poison anaphylatoxin. However, in the later studies of Friedberger, and especially in the work of other authors, the term anaphylatoxin was applied to any poisonous substance which was able to produce in normal animals the phenomena similar to those of anaphylactic syndrome.

Thus various authors have called anaphylatoxin the chemical poisons obtained by Vaughan from bacteria; and Nathan, Bordet, and Mutermilch called anaphylatoxins substances obtained by them from the serum by adsorption with inert substances. The attempts of these authors to explain the nature of anaphylatoxin seem to be generally inadequate, because even though, as my experiments also have confirmed, by digestion of serum with kaolin, for instance, one can produce from serum a substance which is similar in its physiological action, on the one hand, to the anaphylatoxin of Friedberger, and, on the other hand, to the chemical poison of Vaughan, its identity with one or the other is not proved by this similarity alone.

Inasmuch as my experiments show that the cnanges which the serum undergoes under the influence of kaolin are identical with the changes taking place in the immune serum as a result of its interaction with the antigen, they seem to throw light on the nature of anaphylatoxin. Since the process of the formation of toxic products in both cases is established as identical, the similarity of the biological properties of the respective end-products may speak for their identity. These findings, taken in connection with the results reported by me in the previous communication, suggest that the nature of anaphylatoxin is as follows: Fresh serum[3] contains normal proteolytic ferments whose digestive action *in vivo* is inhibited by the simultaneous presence of some antitryptic elements. This antitrypsin can be removed from the serum *in vitro* by two independent processes: one non-specific, a simple mechanical adsorption by means of excess of some organic as well as some inorganic substances; the other specific, an inactivation of the antitryptic properties of the serum taking place as a result of the physicochemical changes in the serum induced by the specific interaction between the antigen and the antibody of the immune serum. The removal of the inhibiting antitryptic action of the serum by either method is followed by the restitution of the activity of the normal proteolytic enzyme, which digests the globulin of the serum.[4] At a certain stage of this autodigestion the serum exhibits toxic properties which are able to cause a typical reaction (local or general) in homologous animals.

SUMMARY.

1. The union of fresh serum of pregnant or immunized animals with the corresponding boiled protein (substratum) is accompanied by the formation of poisonous substances.

[3] Heated serum is free from these ferments unless reactivated by the addition of fresh serum (complement).

[4] It is probable that such proteolytic ferments, activated (by the removal of the inhibition exerted by serum antitrypsin) through the combination of antigen and antibody *in vivo,* may attack also the sensitized circulating antigen. What is important, however, in relation to the recent theories of immunity advanced by Abderhalden, is the fact that such ferments are not specific and when placed in contact with the coagulated antigen *in vitro* are absolutely unable to digest the latter.

2. The poison originates from the serum as a result of its autodigestion, and not from the substratum.

3. The process of autodigestion may be determined by the specific or non-specific removal of the antitrypsin of the serum.

4. The poisons originating from the serum are toxic only for homologous animals.

5. The autodigestion of the serum, if allowed to proceed far enough, may go beyond the toxic stage.

6. The biological properties of these poisons indicate their close similarity to the anaphylatoxin, and suggest that the anaphylatoxin of Friedberger is a product of the autodigestion of serum, and not of the protein outside of the serum.

BIBLIOGRAPHY.

1. Friedberger, E., and Cederberg, O. A., *Centralbl. f. Bacteriol., 1te Abt., Orig.*, 1914, lxxii, 385.
2. Bronfenbrenner, J., *Proc. Soc. Exper. Biol. and Med.*, 1914, xii, 3, 4.
3. Bronfenbrenner, *Jour. Exper. Med.*, 1915, xxi, 221.
4. Doerr, R., *Wien. klin. Wchnschr.*, 1912, xxv, 331.
5. Friedberger, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1909–10, iv, 636.
6. Friedberger, E., and Castelli, G., *Ztschr. f. Immunitätsforsch., Orig.*, 1910, vi, 179.
7. Friedberger, E., and Vallardi, C., *Ztschr. f. Immunitätsforsch., Orig.*, 1910, vii, 94.
8. Vaughan, V. C., and Wheeler, S. M., *Jour. Infect. Dis.*, 1907, iv, 476.
9. Ritz, H., and Sachs, H., *Berl. klin. Wchnschr.*, 1911, xlviii, 987.
10. Bordet, J., *Compt. rend. Soc. de biol.*, 1913, lxxiv, 225.
11. Nathan, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1913, xvii, 478.
12. Mutermilch, S., *Ann. de l'Inst. Pasteur*, 1913, xxvii, 83.
13. Friedberger, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1913, xviii, 227.
14. Bronfenbrenner, *Proc. Soc. Exper. Biol. and Med.*, 1914, xii, 48.
15. Vaughan, V. C., Cumming, J. G., and Wright, J. H., *Ztschr. f. Immunitätsforsch., Orig.*, 1911, ix, 458.
16. Bronfenbrenner, *Proc. Soc. Exper. Biol. and Med.*, 1914, xii, 6, 7.