ON ANAPHYLATOXINS AND ENDOTOXINS OF THE TYPHOID BACILLUS.*

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The endotoxin theory of Pfeiffer, although failing to explain the systemic symptoms of intoxication accompanying diseases like those caused by anthrax bacilli, streptococci, staphylococci, and some other bacteria from the bodies of which no poisonous substances could be obtained, nevertheless furnished an explanation, satisfactory until recently, for the general toxemia accompanying typhoid fever, cholera, and a number of other infections.

In the latter conditions the fact that the injection of dead bacteria or of bacterial extracts, obtained in various ways, had strong toxic action, seemed to indicate the existence of preformed poisonous ingredients in the cell bodies of the bacteria.

The recent work of Friedemann (1), and especially that of Friedberger (2) and his pupils, on the production of non-specific, acutely toxic substances (anaphylatoxins) from cells and bacteria of various kinds, by treatment with specific antibody and complement, has given ample ground for questioning whether the assumption of specific endotoxins, in any of the bacteria, is at all necessary for the comprehension of the bacterial toxemias.

In the light of this work, we may justly assume that the toxic substances may appear only after proteid cleavage of the bacterial bodies has been initiated by the action upon them of the serum components, and that the apparent specificity of the poisons, or differences between the toxemic manifestations of various diseases, may depend not on differences in the pharmacological actions of these poisons, but rather upon variations in the invasive properties

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of the bacteria, both as concerns their quantitative distribution and their accumulation and localization in the infected body.

If we leave out of consideration bacteria which, like the diphtheria bacillus, produce true secretory poisons, it would be the ability to gain a foothold in the body, the degree of invasive power, the predilection in the choice of a path of entrance, and the specific local accumulation, upon which the speed and quantity of toxin production and absorption would depend, and which consequently would give character to variations in the clinical pictures of different diseases. Besides simplifying considerably our comprehension of bacterial toxemia, the point of view suggested by this work again brings out the great importance of the work of Vaughan, and Vaughan and Wheeler (3), on the non-specific poisonous fraction obtained by hydrolysis of bacterial and other proteids, and makes it desirable that the particular conditions of anaphylatoxin and endotoxin production in the case of individual pathogenic bacteria should be carefully studied.

For, even though the existence of anaphylatoxins seems to render the assumption of endotoxins unnecessary, it is still possible that such bodies may exist in addition to the anaphylatoxins, adding a further specific element to the clinical characteristics of each disease.

Though the toxic action of bacteria freshly extracted by such rapid methods as that of freezing and grinding, as practiced by MacFadyen and others, would seem actually to prove the existence of endotoxins, even this method may be interpreted merely as another way of furnishing antigen, or matrix, for anaphylatoxin, since Neufeld and Dold (4) have produced such poisons by the action of fresh serum components on prodigiosus and typhoid extracts.

The purpose of the experiments recorded in the present paper was to study particularly the conditions of anaphylatoxin production from the typhoid bacillus. They are published because, in a number of points, they show results which differ in principle from those obtained for anaphylatoxins in general by Friedberger, Neufeld and Dold, and others, and which, in the writer's opinion, rather strengthen the conception of the manifestations of typhoid fever as being largely dependent upon anaphylatoxin poisoning.

I.

The first series of experiments was carried out according to the earlier technique of Friedberger, in that the bacteria were first sensitized in the cold (3° to 5° C.) for twenty-four hours, then washed and exposed to complement at the same temperature for the same length of time. Injections were then made into the jugular veins of guinea pigs.

The particular point in this first series of experiments was to study the relationship between the degree of anaphylatoxin production and the bactericidal titre (sensitizer content) of the immune sera.

All the experiments were carried out with the same strain of typhoid bacilli (*Bacillus typhosus* 65) and the bactericidal titrations were done by a slightly modified Stern-Korte technique, with fresh rabbit serum as complement, and the usual controls.

These experiments in the main tend merely to confirm the work of Friedberger in showing that when small amounts of bacteria are employed an excess of sensitizer tends to reduce the yield of anaphylatoxin. An example of a successful experiment is given in the following protocol.

TITRATION EXPERIMENT WITH TYPHOID IMMUNE SERUM.

Rabbit 79.

Dilution of serum.	Agglutination.	Bactericidal titre with modified Stern-Korte method.
1 : 100	+++	480 colonies.
1 : 200	+++	556 colonies.
1 : 500	+++	750 colonies.
1 : 1,000	++	Over 10,000 colonies.
1 : 2,000	±	+++++
1 : 5,000	−	+++++
1 : 10,000	−	+++++

0.2 c.c. of this serum added to 1 c.c. of typhoid filtrate gave a very slight clouding in about 15 minutes.

ANAPHYLATOXIN EXPERIMENTS.

No. in series.	Typhoid bacilli.	Amount of inactive serum.	Amount of complement.	Weight of animal.	Result.
1	½ slant	5.0 c.c.	4 c.c.	215 gm.	Very sick, recovers.
2	½ slant	3.5 c.c.	4 c.c.	200 gm.	Typical death in 2 min.
3	½ slant	3.0 c.c.	4 c.c.	198 gm.	Typical death in 2 min.
4	½ slant	2.0 c.c.	4 c.c.	225 gm.	Typical death in 2 min.
5	½ slant	1.0 c.c.	4 c.c.	200 gm.	Sick, recovers.

The above tabulation may be taken as summarizing a considerable number of similar experiments. Throughout our work with low temperature exposures, however, fatal anaphylatoxins were obtained in a few cases only. This, taken together with the work described below, seems to indicate that, in the case of the typhoid bacillus, a more vigorous action of serum upon the bacteria than is obtained at 0° to 5° C. is necessary for the formation of these poisons.

Moreover with the small amounts of bacteria (⅕ to ⅓ agar slants) and the low temperature exposures, we succeeded in no case in producing anaphylatoxin by the action of complement alone.

Because of the weak nature of the poisons produced in most cases, it was now considered advisable to change the technique to one used by Friedberger in his later work, in which the sensitized bacteria were exposed to the action of the complement at 37.5° C. It seemed to the writer that possibly the strong inhibition of complement action by the low temperature might account for the poor yield of poisons, but the higher temperature had been avoided because of the fear that a too vigorous lytic action might prevent the production of anaphylatoxins, as indicated in the experiments of Neufeld and Dold. A chance experiment carried out at this time furnished an indication for the proper direction in which to continue the work.

In the course of experiments planned to remove sensitizer from an inactivated immune serum, the total bacteria of ten agar slants had been left in contact with two cubic centimeters of serum for one hour at 37.5° C. The mixture was then centrifugalized, the serum used for another purpose, and to the bacteria, washed once, were added four cubic centimeters of fresh guinea pig serum. These mixtures were left in the incubator over night, then centrifugalized, and the supernatant fluid was injected into guinea pigs. The experiment was done in duplicate. There were no small pigs in the laboratory at this time and animals weighing 300 and 350 grams had to be used. The results were as follows:

No. in series.	Amount of bacteria.	Immune serum 1 hr. at 37.5° C.	Complement 12 hrs. at 37.5° C.	Weight of guinea pig.	Result.
1	10 slants	2 c.c.	4 c.c.	300 gm.	Typical death in 2 min.
2	10 slants	2 c.c.	4 c.c.	350 gm.	Typical death in 4 min.

This experiment indicated definitely that, in the case of typhoid bacilli, at least, comparatively large quantities of bacteria could be used without inhibiting the production of anaphylatoxin, a result in contrast with some of Friedberger's experiments, and that exposure to conditions, under which continued action of the complement could take place for prolonged periods of time (twelve hours), did not lead to negative results.

Since Neufeld and Dold found that no anaphylatoxin was obtained when 2 c.c. of complement were allowed to act upon three loops of sensitized cholera spirilla for but one and a half hours at 37.5° C., and concluded from this that in the course of actual lysis the anaphylatoxins were either hindered in appearance or destroyed, our apparently contradictory result called for further investigation.

The fact that powerful anaphylatoxin could be produced from sensitized typhoid bacilli, even when large amounts of bacteria were used and the exposure to complement was continued for as long as twelve hours at 37.5° C., seemed to indicate that in the case of this microörganism there was either a slower, cumulative formation of the poison or that, once formed, it was more stable and not as easily further disintegrated ("abgebaut") into non-toxic substances. This would, in our opinion, tend to throw more weight upon the assumption that anaphylatoxins were responsible to a large extent for the toxemic manifestations of typhoid fever, where, as we know, considerable quantities of bacteria are exposed to the prolonged action of high concentrations of the specific antibodies at temperatures of from 37.5° to 40° C.

In order to study further these relations the following experiments were carried out. In both experiments the bacteria were grown for twenty-four hours on agar slants, taken up in salt solution, and when sensitized, washed once before the addition of complement.

Comparing the results of the experiments summarized in these two protocols with those reported in the first part of this paper we see that the exposure of bacilli to the action of serum constituents at a temperature of 37.5° C. is, at least in the case of *Bacillus typhosus,* a much more efficient and regular method of obtaining the

anaphylatoxin than is the original method of allowing this interaction to take place at low temperatures.

EXPERIMENT I.

The materials used were *Bacillus typhosus* 65, typhoid immune rabbit serum (from rabbit A)[1] inactivated at 56° C., and fresh guinea pig serum as complement. The injected guinea pigs weighed from 150 to 225 grams.

No. in series.	Amount of bacteria.	Sensitized with inactive serum 1 hr. at 37.5° C.	Time of exposure to complement at 37.5° C.	Amount injected.	Result.
1	½ slant	1 c.c.	1 hr.	4 c.c.	Very slightly sick, recovers.
2	1 slant	1 c.c.	1 hr.	4 c.c.	Distinct symptoms, recovers.
3	1 slant	Not sensitized	1 hr.	4 c.c.	Distinct symptoms, recovers.
4	1½ slants	1 c.c.	1 hr.	4 c.c.	Typical death in 2 min.
5	2 slants	1 c.c.	1 hr.	4 c.c.	Typical death in 4 min.
6	9 slants	1 c.c.	1 hr.	4 c.c.	Typical death in 5 min.
7	9 slants	Not sensitized	1 hr.	4 c.c.	Typical death in 5½ min.
8	12 slants	1 c.c.	1 hr.	4 c.c.	Very sick, recovers.
9	12 slants	Not sensitized	1 hr.	4 c.c.	Very slightly sick, recovers.

EXPERIMENT II.

The materials used and the technique of the experiment were the same as those in experiment I.

No. in series.	Amount of bacteria.	Sensitized with inactive serum 1 hr. at 37.5° C.	Time of exposure to complement at 37.5° C.	Amount injected.	Result.
1	½ slant	1 c.c.	15 hrs.	4 c.c.	Very sick, recovers.
2	1 slant	1 c.c.	15 hrs.	4 c.c.	Typical death in 8½ min.
3	2 slants	1 c.c.	15 hrs.	4 c.c.	Typical death in 5 min.
4	2 slants	Not sensitized	15 hrs.	4 c.c.	Typical death in 5½ min.
5	3 slants	1 c.c.	15 hrs.	2.5 c.c.	Typical death in 4 min.
6	3 slants	4 c.c.	15 hrs.	4 c.c.	Typical death in 4 min.
7	3 slants	Not sensitized	15 hrs.	4 c.c.	Typical death in 2 min.
8	8 slants	1 c.c.	15 hrs.	4 c.c.	Typical death in 5 min.
9	8 slants	Not sensitized	15 hrs.	4 c.c.	Typical death in 4 min.
10	12 slants	1 c.c.	15 hrs.	4 c.c.	Very sick, lives.
11	12 slants	Not sensitized	15 hrs.	4 c.c.	Slightly sick.

The most important and interesting point brought out in these two protocols, however, it seems to us, is the fact that, with approximately equal quantities of bacteria, exposure to the serum for as long as fifteen hours at 37.5° C. not only does not destroy the

[1] This serum had an agglutinating titre of 1:8,000 for *Bacillus typhosus* 65.

anaphylatoxins once formed, but gives more powerful results than in the shorter periods of exposure. Thus guinea pigs 2 and 3 of experiment I recovered, whereas guinea pig 2 of experiment II died with typical symptoms in eight and a half minutes. In this, as in subsequent work, we have found that, with typhoid bacilli, the method of prolonged exposure gives powerful poisons with unfailing regularity. In the case of *Bacillus typhosus,* therefore, the anaphylatoxins are not as unstable and amenable to destruction by serum proteolysis as in the case of some other bacteria investigated by Neufeld and Dold.

When quantities of bacteria as large as twelve slants (or more in a few untabulated experiments) were used without sensitization or with moderate sensitization, no poisons were formed. This is in accord with the experiments of Friedberger and others with shorter exposures at 37.5° C., and with the low temperature method.

Although we cannot fully explain this point, it would seem that possibly this could be taken to support the view expressed by Bordet (5) that the distribution of serum substances upon an antigen is such that the entire amount of antibody is distributed equally among the antigenic elements, and, in the case of an excess of bacteria, as in our experiments, the quantity falling to each unit is insufficient, at least in the time of exposure here practiced, to accomplish the cleavage necessary for poison production.

It would appear from these experiments, moreover, that within a range of from no sensitization to that of sensitization of three slants with four cubic centimeters of antiserum, there is no difference in anaphylatoxin production, either in the case of exposures to 37.5° C. for one hour, or in the more prolonged experiments.

This would be, if true, a contradiction of the results of Friedberger and Szymanowski's experiments with *Vibrio metchnikovi,* which showed that, while anaphylatoxins could be produced by the action of complement upon both the sensitized bacteria and the unsensitized, the process was considerably slower in the latter case. Since the experiments of these observers were carried out with quantities of four loopfuls of the vibrios, it seemed desirable that experiments should be carried out by us with typhoid bacilli in the larger quantities used in our experiments, in order that we might

determine especially whether or not the sensitizer took any part in the production of anaphylatoxin in the method pursued by us.

Experiments were done with this point in view and it was found that, when three slants of typhoid bacilli were used, fatal anaphylatoxins could be produced from sensitized bacilli in forty minutes at 37.5° C., while it required an hour or longer to procure similar results from the unsensitized bacteria. Since this merely confirms the results of Friedberger and Szymanowski the protocols of our experiments are omitted.

There is not a fundamental difference between the action of complement on a bacterial antigen with and without the coöperation of a specific antibody, if we consider that complement in small amounts may be fixed by bacterial emulsions of various kinds. Whether this is accomplished by the aid of small amounts of specific sensitizer normally present in the guinea pig serum, or by reason of peculiarities of the bacterial proteid which, differing from some other cellular antigens, is capable of adsorbing complement independently of the sensitizer, the fact remains that we have here a capacity of bacteria for entering into relationship with complement, and the addition of specific immune bodies merely increases this capacity and hastens its action. And this conception does not appear to us affected by the doubt recently thrown upon the identification of complement fixation with complement action by the work of Liefmann and Cohn (6); for while the binding of complement is there conceived not as preceding hemolysis (and by analogy bacteriolysis), but as resulting from the liberation during this process of complement-fixing products, even in such a case the actual fixation would be an indication of a reaction having taken place in which complement has actively functionated.[2]

II.

It was a part of the plan of this work to compare the poisonous properties of the typhoid anaphylatoxins prepared as described above with the toxic action of the substances obtained from typhoid bacilli by methods hitherto spoken of as endotoxin extraction. It

[2] In connection with the work reported in this paper we have attempted to repeat the experiments of Keysser and Wassermann (7) who obtained anaphyla-

seemed also important to carry out, in such a comparative study, experiments parallel to those described in the recent work of Rosenow (8), who succeeded in obtaining substances similar to anaphylatoxins in their action upon guinea pigs, from pneumococci, typhoid bacilli, and other bacteria by a process probably of autolysis.

For these reasons the following experiments were carried out in the course of our work on typhoid anaphylatoxins.

INJECTION INTO GUINEA PIGS OF WHOLE TYPHOID BACILLI KILLED AT 60° C.

The organisms, *B. typhosus* 65, were grown on agar slants. They were then taken up in salt solution, heated to 60° C. for one hour in a water bath and immediately injected intravenously. The following table is typical of the results obtained.

No. in series.	Amount injected.	Weight of guinea pig.	Result.
1	1 slant	210 gm.	No immediate symptoms; dead in 2 dys.
2	1 slant	225 gm.	No immediate symptoms; lives.
3	2 slants	195 gm.	No immediate symptoms; dead in 26 hrs.

EXPERIMENTS WITH CULTURE FILTRATES.

1. *B. typhosus* 65 was cultivated in 0.3 per cent. alkaline broth for ten days and then filtered. 5 c.c. of this filtrate injected into a rabbit of 1,350 gm. produced diarrhea (feces streaked with blood), weakness, paralysis of hind legs, and death during the night, after about 16 to 24 hours.

Experiment with Guinea Pigs.

No. in series.	Intravenous injection.	Weight of guinea pig.	Result.
1	4 c.c.	225 gm.	No symptoms after injection. Diarrhea. Dead in 48 hrs.
2	5 c.c.	250 gm.	Dead in 72 hrs.
3	5 c.c.	265 gm.	Very sick after 2 dys; recovers.

2. *B. typhosus* 65 was cultivated in alkaline broth for two months and then filtered. 5 c.c. of this filtrate produced diarrhea and slight illness in a rabbit of 1,500 gm., but did not kill it.

toxins by allowing complement to act upon kaolin and barium sulphate previously sensitized by exposure to inactivated normal and immune sera. We have so far been unable to confirm their results, being forced to attribute the moderate symptoms resulting in some of our experiments to the similar action of slight amounts of kaolin injected with the serum. Comparative titration, moreover, did not indicate that, as claimed by the above writers, kaolin emulsions may absorb sensitizer or amboceptor out of concentrated inactivated serum.

Experiment with Guinea Pigs.

No. in series.	Intravenous injection.	Weight of guinea pig.	Result.
1	5 c.c.	240 gm.	Slight immediate illness; recovery.
2	4 c.c.	180 gm.	Slight immediate illness; recovery.

EXTRACTION OF TYPHOID BACILLI BY MECHANICAL METHODS.

1. The bacilli from twelve agar slants were emulsified in 25 c.c. of salt solution, heated to 60° C. for one hour, then placed in a shaking machine in a bottle with sterile glass beads and shaken for forty-eight hours at room temperature. The emulsion was then centrifugalized until most of the bacterial bodies had been thrown down, and the supernatant fluids were injected intravenously. The results were as follows:

No. in series.	Amount injected.	Weight of guinea pig.	Result.
1	4 c.c.	200 gm.	No immediate symptoms; survives.
2	4 c.c.	200+ gm.	No immediate symptoms; survives.

2. Bacilli from twenty agar slants were taken up in 10 c.c. of salt solution and centrifugalized until almost all had been thrown down. The bulk of the sediment, having a volume of 1.5 c.c., was freed from all supernatant fluid with pieces of blotting paper, and to it was added 0.45 gm. of sodium chloride in the centrifuge tube. This formed a paste which became very thick and gritty when placed in a beaker containing a mixture of brine and ice, in which state it was ground with a glass rod for one hour. Sterile distilled water was then added to give a total volume of 50 c.c., which gave a concentration of 0.9 per cent. of salt. This was heated to 65° C. for one hour and then injected. The opalescent liquid resulting from this treatment contained many well preserved bacilli but, with them, a large number of swollen and granular, poorly staining shadow forms, indicating that many of the bacteria had become disintegrated.

The results of injection were as follows:

No. in series.	Amount injected.	Weight of guinea pig.	Result.
1	3 c.c.	200 gm.	Very sick after injection but symptoms not like those of anaphylatoxin. Cowers quietly, fur ruffled, rapid breathing. Dead in 8 hrs.
2	2 c.c.	110 gm.	Same as above. Dead in 6 hrs.

EXPERIMENTS WITH TYPHOID BACILLI SUSPENDED FOR SHORT PERIODS IN NEUTRAL OR INACTIVE LIQUIDS (AUTOLYSATES (?)).

During the course of the immunization of rabbits with typhoid bacilli, an accident occurred which, in conjunction with the work

of Neufeld and Dold, and the similar studies of Rosenow, encouraged us to investigate the toxic properties of typhoid bacillus autolysates. It had been our habit to begin the immunization of rabbits with injections of one half an agar slant of bacilli heated to 60° C. for one hour. The material for such an injection had been prepared and, because of interruption, was allowed to stand at room temperature over night, instead of being injected at once, as was usually done. On the following morning, two unusually large Belgian hares, weighing 3,000 grams, were injected intravenously and became severely sick, dying within twenty-four hours. Since much smaller rabbits had repeatedly withstood, without symptoms, these and larger doses of dead typhoid bacilli of strain 65, we were at first at a loss to explain the death of these animals, until, upon reading the experiments of the workers quoted above, the possibility of autolytically produced toxins suggested itself.

In consequence it seemed advisable, in connection with our work on anaphylatoxins, to study upon guinea pigs the poisonous properties of substances formed in salt solution emulsions of typhoid bacilli. The following protocols illustrate these experiments.

EXPERIMENT I.

B. typhosus 65, grown for twenty-four hours on agar slants, was emulsified in salt solution in proportions of one slant to 4 c.c. of salt solution. The emulsions were kept in the incubator in centrifuge tubes, and, at varying times as indicated, one of them was taken out, most of the bacilli were thrown down, and the supernatant liquid was injected intravenously into guinea pigs with the following results.

No. in series.	Length of time at 37.5° C.	Amount injected.	Weight of guinea pig.	Result.[3]
1	1 hr.	4 c.c.	200 gm.	No immediate symptoms.
2	2 hrs.	4 c.c.	195 gm.	Slight respiratory distress.
3	6 hrs.	4 c.c.	215 gm.	No symptoms.
4	8 hrs.	4 c.c.	225 gm.	Convulsive twitching, great respiratory distress, falls to side; recovers.
5	8 hrs.	4 c.c.	210 gm.	Slightly less than preceding.
6	24 hrs.	4 c.c.	250 gm.	Very slightly sick.

Experiments I and II were not carried out with the purpose of making an extensive study of the so called typhoid endotoxins.

[3] A number of these guinea pigs died later owing to injection of living bacteria incompletely thrown down in centrifugation.

EXPERIMENT II.

Four agar slants of *B. typhosus 65* were emulsified in 12 c.c. of salt solution. They were then divided into 3 parts of 4 c.c. each and each part was shaken up with 1 c.c. of ether, and placed at 37.5° C. for varying times, as indicated.

No. in series.	Length of time at 37.5° C.	Amount injected.	Weight of guinea pig.	Result.
1	2 hrs.	4 c.c.	240 gm.	Very slightly sick.
2	3 hrs.	4 c.c.	225 gm.	Very slightly sick.
3	4½ hrs.	4 c.c.	210 gm,	Very sick, convulsive movements, weak, falls on side, difficult respiration; recovers.

but simply in order to furnish a basis for comparison between the action of these bodies and the anaphylatoxic substances. In none of the animals in which death resulted from injections of the typhoid extracts were either the symptoms or the necropsies in any way comparable to those occurring in the cases of anaphylatoxin poisoning. None of the former showed the characteristic inflation of the lungs invariably found in the latter. Moreover, in animals dying of the extracts, hemorrhages into the serous surfaces, chiefly the peritoneum, and into the wall of the large intestine, were not infrequent, and these animals often had diarrhea before death. This was especially the case in animals dying of injections of the more powerful poisons produced by the modification of Besredka's method in which the bacilli had been ground with salt before extraction. Even in these cases, however, the clinical picture was very different and more protracted than in the cases of fatal anaphylatoxin poisoning.

Now it by no means follows that this is evidence of a fundamental difference between the two modes of poisoning, since the injection of any or all of the typhoid extracts might be rationally interpreted merely as the furnishing of a matrix, or antigen, for the production of anaphylatoxin by the blood constituents of the animal after their introduction.

The slower death from these extracts might then be looked upon as a gradual anaphylatoxin poisoning, progressive and cumulative, which would be likely to differ, both in symptomatology and pathology, from that of the more sudden and acutely fatal anaphylatoxin poisoning. To determine this point it would be desirable to experi-

ment upon the results of the frequent administration of small quantities of anaphylatoxin over a prolonged period. On this point we have, at present, no completed observations.

SUMMARY AND CONCLUSIONS.

The experiments recorded in this paper confirm the observations of Friedberger that acutely toxic bodies can be produced from typhoid bacilli by the action of sensitizer and complement and that, when small quantities of bacteria are used, an excess of sensitization either interferes with the formation of the poisons or leads to a cleavage of the bacterial proteid beyond the poisonous intermediate products spoken of as anaphylatoxins. Unlike the experience of other workers with poisons of this nature, however, our experiments have shown that the action of complement upon typhoid bacilli strongly sensitized or not at all sensitized may be carried on, at body temperature, for considerably longer than twelve hours without leading to a destruction of the poisons, and that this is true when the quantities of the bacteria used vary within the wide range of from one to twelve agar slants. It has been found, in fact, that in the case of this microörganism prolonged exposure at the higher temperature of considerable quantities of bacteria constitutes an unfailing method of regularly obtaining powerful poisons. The results obtained by the use of smaller quantities and the less vigorous complement action at low temperatures are far less regular or satisfactory.

It would appear from this that complement action of considerable vigor is required to obtain from this bacillus any appreciable yield of anaphylatoxin, and that the poison, once formed, is not as unstable as that found in other microörganisms by Neufeld and Dold and others. In fact, although we have never observed complete lysis *in vitro* of the typhoid bacilli treated with antibody and complement, the sensitized bacteria exposed to the action of complement for as long as fifteen hours at 37.5° C. showed, in our experiments, much disintegration, and yet powerful poisons were present.

Were the influence of lysis or of the too vigorous action of the serum bodies as rapidly poison-destroying in the case of this

bacillus as it has been shown to be in the case of some other bacteria, it would be hard to understand how anaphylatoxins could play any part in the toxemia of typhoid fever. This phase of our experiments, however, seems to indicate that the conditions prevailing in the infected body at the height of this disease would furnish ideal criteria for anaphylatoxin production, since, in such cases, vigorously sensitized bacilli, in large numbers, are under the prolonged influence of considerable quantities of complement, conditions exactly comparable to those prevailing in our experiments.

Granted that this state of affairs is actually the case, then the subsidence of the disease might depend merely upon limitation of the supply of antigen, as the increasing bactericidal action of the blood constituents come into play, and upon the consequent diminution of the anaphylatoxin. For as the bacteria diminish and the sensitizer increases, a changed proportion between them is established which, finally, as experiment has shown, results in a failure of anaphylatoxin production. For although our experiments have shown that, within a wide latitude of relative proportions of bacteria and antibody, anaphylatoxin can be formed, beyond this range an excess of one or the other element eventually will prevent their formation. It is not, however, the purpose of this paper to discuss the mechanism of the subsidence of the disease since this phase of the work will necessitate further experimental study.

In regard to the experiments with kaolin, we were unable to confirm the contention of Keysser and Wassermann, though it is more than likely that toxic bodies could be formed by the action of complement upon any foreign proteid rendered amenable to its action. We are not inclined to attribute too much importance to these negative results, recording them merely as they occurred. However, should it be found subsequently that anaphylatoxins can be formed in this way, it seems unlikely that they are formed from the sensitizer or amboceptor as matrix, since this was not specifically adsorbed out of concentrated serum by the kaolin in our experiments.

On the basis of experiments with so called endotoxins, we feel that the existence of such preformed intracellular poisons as an element in typhoid toxemia has not been proved, and is not abso-

lutely necessary for the explanation of the phenomena occurring in this disease. However, the diarrhea, the hemorrhagic lesions, and the protracted symptoms following the injection of extracts and filtrates of the bacillus, differing so strikingly from the acute illness with rapid death or equally rapid recovery resulting from anaphylatoxin poisoning, would justify the assumption that poisons of this nature may still play a part in the disease, adding an additional specific characteristic to the clinical picture. As stated before, however, it is not improbable that all these characteristics may represent merely a more protracted or subacute state of anaphylatoxin toxemia.

The experiments with autolysates, although none of them were fatal in their results upon guinea pigs, have sufficiently indicated that poisons comparable to anaphylatoxins can be formed in this way. This would indicate that a reaction of proteolysis, which may take place slowly by autolysis, is hastened by the action of complement, and its velocity is still further augmented by the increase, within certain limits, of the sensitization,—a conception which would attribute to the combined action of complement and sensitizer a function not incomparable to that of the bodies spoken of as catalytic agents.

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