STUDIES ON BACTERIAL ANAPHYLAXIS AND INFECTION.

By HANS ZINSSER, M.D., and JULIA T. PARKER.

(From the Department of Bacteriology af the College of Physicians and Surgeons, Columbia University, New York.)

PLATES 38 AND 39.

(Received for publication, April 19, 1917.)

Since anaphylaxis has now been clearly recognized as a phenomenon which depends upon an antigen-antibody reaction, it follows naturally that the fundamental principles underlying these manifestations should apply, subject perhaps to individual modifying factors, to all substances which on injection into the animal body induce specific antibody formation. By far the greater volume of work on anaphylaxis has been carried out with antigens consisting of animal sera, egg white, and other freely suspended proteins not enclosed in cells. And in the case of such serum anaphylaxis it has been possible to go far in the analysis of the mechanism of the phenomenon and its application to such practical problems as serum sickness and other clinical conditions referable to abnormal protein metabolism.

There has been relatively less progress, however, in the study of the hypersusceptibility incident to the treatment of animals with cellular antigens; that is, with bacteria, erythrocytes, and other cells. And yet these phenomena in the case of bacteria may reasonably be assumed to play an important part in the symptomatology and pathology of infectious diseases where there is a continuous liberation of fluctuating amounts of specific antigen in a body at first perhaps unsensitized, but later increasingly hypersusceptible, and perhaps repeatedly sensitized and desensitized during the course of the disease. Of these conditions we know very little, and correspondingly much speculation and reasoning from the analogies of serum anaphylaxis have been the bases of many inferences and theoretical suggestions.

It is plain that when the antigen employed is a cellular one we are necessarily dealing with a phenomenon consisting of two phases. As integral parts of compact structures, the cell constituents cannot react as antigen with the cells and fluids of the invaded body until they have been liberated by cell disintegration. After that the process may be, and probably is, entirely similar to serum anaphylaxis. But before this state of affairs can be brought about it must be assumed that some preliminary reaction occurs in which the bacterial protein is set free from the cell. As to the nature of this reaction we have no certain knowledge. We cannot be sure at present, at least in our opinion, whether such a reaction is or is not accompanied by the direct formation of toxic cleavage products within the circulation such as those produced *in vitro* by Vaughan,[1] Friedberger,[2] and others, though this seems likely. Moreover, we do not know to what degree and under what circumstances such a process may be interfered with by intercurrent phagocytosis, and perhaps by bacterial agglutination and accumulation in the viscera, as demonstrated by Bull.[3]

The practical bearing of bacterial anaphylaxis on the course and symptomatology of infectious disease is of necessity very great. It has been suggested that the incubation time of infections may be in part dependent upon the gradual development of the specific sensitization of the tissues as antibodies begin to appear. The evidence adduced by the study of the isolated uterus, intestinal musculature, the perfused heart and lungs, and such experiments as those of Longcope[4] on the kidneys of rabbits points to the possibility that many of the insults offered various tissues, formerly attributed to specific preformed bacterial poisons, may be anaphylactic in nature. It is not impossible that a process of alternate sensitization and desensitization, either interrupted or continuous and gradual, may represent the dominating mechanism of injury in bacterial disease.

[1] Vaughan, V. C., Vaughan, V. C., Jr., and Vaughan, J. W., Protein split products in relation to immunity and disease, Philadelphia and New York, 1913.

[2] Friedberger, E., Series of papers in *Z. Immunitätsforsch., Orig.*, 1910 to 1913.

[3] Bull, C. G., *J. Exp. Med.*, 1915, xxii, 475.

[4] Longcope, W. T., *J. Exp. Med.*, 1913, xviii, 678.

We do not yet understand clearly the relation of the circulating antibodies to such processes. Do they aid in the intravascular mechanism for the liberation of toxic substances as suggested by Pfeiffer, Radziewsky, Wolff-Eisner, and more recently by Vaughan, Friedberger, and others? Or do they both hasten the removal of antigen by bactericidal and opsonic action and perhaps even protect the sensitized tissues by combining with the circulating antigen and diverting it from the sessile antibodies of the sensitized tissues? It would be interesting and important to know, moreover, whether or not there is a true bacterial hypersusceptibility in the sense that an animal treated with dead bacteria or bacterial products (as in vaccination) might pass through a preliminary period during which susceptibility to invasion by the living organisms or injury by their body constituents is increased.

All these problems await an analysis of bacterial anaphylaxis as thorough as that which has been made with antigens of non-cellular nature.

In order to approach the problem properly it is desirable to review briefly what has been done and how much of it may be accepted as needing no further inquiry.

The first investigators who systematically studied serum anaphylaxis naturally turned their attention, also, to anaphylaxis produced with bacterial protein. Thus Rosenau and Anderson[5] obtained definite results with extracts of various bacteria, using chiefly colon bacilli, extracts of yeast, of *B. subtilis*, and of typhoid bacilli. They injected relatively large quantities of the extracts subcutaneously into guinea pigs and, after periods of from 19 to 35 days, reinjected intraperitoneally or subcutaneously. They obtained reactions which they characterized as "mild," "marked," or "severe," but rarely observed acute anaphylactic death. Subsequently a large number of investigators turned their attention to the same problem and more or less divergent results were reported. The work was done chiefly by Kraus and Doerr,[6] Kraus and Amiradžibi,[7] Holobut,[8] Yamanouchi,[9] Delanoë,[10] Ascoli,[11] and Friedberger and his associates. It is hardly necessary

[5] Rosenau, M. J., and Anderson, J. F., *Bull. Hyg. Lab., U. S. P. H., 36*, 1907.

[6] Kraus, R., and Doerr, R., *Wien. klin. Woch.*, 1908, xxi, 1008.

[7] Kraus, R., and Amiradžibi, F. S., *Z. Immunitätsforsch., Orig.*, 1909–10, iv, 607.

[8] Holobut, T., *Z. Immunitätsforsch., Orig.*, 1909, iii, 639.

[9] Yamanouchi, T., *Compt. rend. Soc. biol.*, 1909, lxvi, 531.

[10] Delanoë, M. P., *Compt. rend. Soc. biol.*, 1909, lxvi, 207, 252, 348, 389.

[11] Ascoli, M., *Compt. rend. Soc. biol.*, 1908, lxv, 611.

to go into a detailed historical review of the literature since the difficulties encountered, and the general conclusions drawn, may be summarized very simply. Most of the work done was carried out with definite prejudice in favor of complete analogy between serum hypersensitiveness and bacterial anaphylaxis. Kraus and Doerr, as well as Holobut and others, claimed that sensitization could be accomplished with regularity only if small doses of bacteria, *i.e.*, one one-hundredth of a loopful, were given daily for 10 days and reinjection was practiced about 3 weeks after the last injection with a relatively large amount washed up with and partly extracted in slightly alkalinized salt solution. It was found by a number of observers that the results were not uniform with different species of bacteria. It apparently has been very difficult, according to Müller, to sensitize with streptococci perhaps, as Müller suggests, because of the relative insolubility of these bacteria. Moreover, acute symptoms on reinjection in bacterial anaphylaxis have not been obtained with regularity and have been comparable to the acute shock in serum anaphylaxis in the smaller number of cases only.

Delanoë has denied the strict specificity of bacterial anaphylaxis, claiming that animals sensitized with typhoid bacilli reacted distinctly when reinjected with colon and paratyphoid A and B bacilli. This, however, may be true and yet fail to contradict the essential specificity of bacterial anaphylaxis as asserted by most investigators, since a group reaction might easily have accounted for his results, together with the toxic reaction so often noticed on first injection of any bacterial protein into guinea pigs.

Passive sensitization against bacterial protein was first reported by Kraus and Doerr and was further worked upon by Kraus and Amiradžibi. They injected guinea pigs with the serum of immune guinea pigs and rabbits, and 24 hours later injected the antigen intravenously or intraperitoneally, obtaining acute shock which sometimes killed the animals. Delanoë reported similar experiments and Yamanouchi and Ascoli suggested the utilization of passive anaphylaxis for the diagnosis of tuberculosis and typhoid fever.

Kraus and Amiradžibi also published experiments in which they obtained shock in guinea pigs into which they had injected typhoid bacilli mixed with the immune rabbit serum just before injection. These experiments are cited throughout the literature as pointing to the intravascular mechanism of bacterial anaphylaxis. Whatever may be our opinion concerning this question, and we will further discuss this in another place, the experiments of Kraus and Amiradžibi are inconclusive since they entirely disregard the not infrequent toxicity of normal and immune rabbit serum for guinea pigs, a disturbing factor which we encountered very early in our own work. In their protocols they record positive results only when the dose of serum was equal to or exceeded 0.5 cc. of rabbit serum and they confirmed the suspicion that serum toxicity was the true reason for the observed shock by stating that the active rabbit serum had lost its ability to give reactions when injected with bacteria after having been pre-

served for a month, a fact which our own investigations and the work of others have shown to be the case with sera toxic for animals of another species. The experiments of Kraus and Amiradžibi therefore, may be disregarded as having any bearing on the intravascular nature of bacterial anaphylaxis.

Nevertheless, from the work of other observers (Friedberger and Mita, Delanoë, and others) it seems beyond question that, just as one would expect, passive sensitization of guinea pigs with the sera of animals sensitized or immune to bacteria is possible. There is some discrepancy, however, between the results obtained by us and those reported by the observers mentioned in regard to the time necessary between injection of the antiserum and the development of the hypersensitiveness. This is a matter concerning which we will have more to say below.

As far, then, as reaction with the dissolved bacterial antigen is concerned there seems to be a complete analogy between serum and bacterial anaphylaxis except in one important and perhaps fundamental point, and this is the fact of primary toxicity of the bacterial protein. For, while the first inoculation of such antigens as horse serum, sheep serum, egg white, etc., produces no symptoms of injury in the injected animal, typhoid protein and that of many other bacteria if given in sufficient amounts lead even at first injection to severe illness and rapid or delayed death according to the variety of bacteria and the amount injected.

Indeed it is an observation recorded by Friedberger and Mita, by Seitz,[12] by Müller,[13] and by Doerr,[14] as well as occasionally made in our own experiments, that typical anaphylactic symptoms may follow immediately upon first injection of bacteria and bacterial extracts, if the amount given is sufficient. Müller particularly, has subjected this occurrence to systematic study. He finds that the chief difference between the reaction of the sensitized and the unsensitized guinea pig to intravenous injection of bacterial protein consists in the facts that the sensitized animal will show acute reaction to doses smaller than those necessary to produce the same result in the normal controls and that these smaller doses, which produce acute anaphylactic death in the sensitized, will produce a

[12] Seitz, A., *Z. Immunitätsforsch., Orig.*, 1912, xiv, 91.

[13] Müller, P. T., *Z. Immunitätsforsch., Orig.*, 1912, xiv, 426.

[14] Doerr, R., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, ii, 1098.

more protracted illness and often death after the lapse of hours or days in the unsensitized. Müller interprets his work entirely in the sense of Friedberger's anaphylatoxin theory, in that he believes acute death to be due to the formation in the blood stream of a poison resulting from the reaction between plasma and bacteria, a process which is therefore more rapid in the sensitized animals in whose blood stream antibodies are present in relatively higher concentration.

These considerations bring the problem of bacterial anaphylaxis into intimate relationship with questions concerning the nature of the toxic properties of such microorganisms as the typhoid, paratyphoid, and plague bacilli, the cholera spirillum, and other bacteria, which unlike diphtheria and tetanus, do not secrete true toxins or exotoxins. Friedberger's theory would both explain anaphylactic poisoning with bacterial antigens and relegate to the chapter of discarded theories the older endotoxin conception of Pfeiffer which assumed the existence of specific preformed intracellular poisons liberated mechanically by disintegration of the cell body.

As a matter of fact, the entire problem of endotoxins is one which calls for reexamination in that the knowledge gained of recent years has opened a number of alternative explanations for the primary toxicity of such bacteria as the typhoid bacillus. Briefly summarized they are: (1) The actual intracellular existence of specific endotoxins in the sense of Pfeiffer[15] (toxalbumin). (2) The production of toxic split products in the animal body from the bacterial protein by proteolytic cleavage brought about by non-specific serum protease (Jobling and Petersen[16]) or by the cooperation of antibody and alexin (Friedberger). (3) The absorption of antienzymes by the bacteria with consequent activation of the serum protease which then splits off toxic substances from the plasma protein (Jobling and Petersen[17]). (4) The presence of non-specific toxic substances in the bacterial cell body, of the nature of peptones, primary and secondary albumoses, etc., which are liberated by lysis from the bacterial cell after cell death. This conception would differ from that of Pfeiffer in that the intracellular substances are conceived as in no sense specific toxic proteins, but rather entirely non-specific constituents repre-

[15] Pfeiffer, R., *Z. Hyg.*, 1892, xi, 393.

[16] Jobling, J. W., and Petersen, W., *J. Exp. Med.*, 1914, xx, 452.

[17] Jobling and Petersen, *J. Exp. Med.*, 1914, xx, 321.

senting the type of poisons conceived as proteolytically produced from the antigen by Vaughan and others. This last view, though hitherto not particularly considered, should nevertheless in our opinion be regarded as at least a possible explanation for a part of the toxic manifestations resulting from the injection of bacteria of this class. Moreover, such a possibility is suggested by the fact that bacterial protein is relatively poor in antigenic properties. Doerr,[18] also, has considered this in stating that he believed the difficulty of producing anaphylaxis with bacteria was in part due to the fact that their body substances were relatively poor in coagulable (antigenic) proteins. We have not been able as yet to study the matter extensively, but we have carried out a few experiments, as follows:

Typhoid bacilli from twenty-four agar cultures were weighed as a moist mass, ground up with salt, and then taken up in distilled water to isotonicity. After the addition of 0.2 cc. of $\frac{N}{1}$ sodium hydroxide to 100 cc. the suspension was heated to 60°C. for 30 minutes to prevent autolysis, and was then shaken for 4 to 5 hours with a motor, at room temperature. After filtration through a Berkefeld candle the clear solution gave a definite cloud after boiling and adding acetic acid. The filtrate was treated with heat and acid to remove coagulable protein. On the advice of Professor Gies the suspension was first brought to a boil and then small amounts of acid were added to prevent possible hydrolysis which might have occurred had the acid been added first. The filtrate from this was then half saturated with ammonium sulfate. Again a definite cloud was obtained, and when this was filtered clear, a second turbidity could be produced by complete saturation with the sulfate.

Although we have not yet obtained toxic reactions with these substances after isolation, perhaps because of the difficulty of obtaining them in sufficient amount, the presence of albumoses, substances which have often been found to possess primary toxicity for animals, suggests the possibility that their existence in the bacterial body might indeed contribute to the injury done by injections of bacteria. We found albumoses in extracts of typhoid bacilli not

[18] Doerr,[14] p. 1100.

only when the bacteria were grown on the ordinary peptone media but also on agar made without peptone to which nutrose or sodium caseinate has been added as an enriching substance.

Moreover, we have also had the experience with the typhoid bacillus which is referred to repeatedly by Vaughan when dealing with the colon bacillus; namely, that bacterial suspensions subjected to boiling are quite as toxic and often more so than are the unheated and living suspensions.

Thus, if three guinea pigs are injected with equal amounts of typhoid suspension, one with the living bacteria, the second with bacteria heated to 60°C. for 15 minutes, and the third with bacteria boiled for 5 minutes, the guinea pig receiving the boiled bacilli will often be the first one to grow sick and may die several hours before the others.

This may mean, of course, as Vaughan suggests, that the heated protein is more promptly split by the ferments of the body. It also suggests, however, that in addition to this the heating has left unchanged non-coagulable toxic constituents of the cell.

Passive Sensitization of Guinea Pigs to Bacteria.

Since in this work we were concerned primarily with the fundamental problems referred to in our introduction, we neglected for the present the purely technical difficulties connected with active sensitization and employed entirely the method of passive sensitization in which a more immediate and direct control over the relationship between the development of hypersensitiveness and the concentration of circulating antibodies could be exercised.

The experiments described in the following paragraphs illustrate the passive sensitization of guinea pigs by intraperitoneal injection with antityphoid serum. They show that unlike Kraus and his collaborators, we never succeeded in finding the animals sensitized in less than 3 to 5 days, the highest degree of sensitization being developed in about a week. This was true both when the whole animal was tested and when the reactions were obtained with the isolated uteri.

Passive Sensitization to Typhoid Protein. (*a*) *Experiment 1.*—Apr. 8, 1916. Guinea Pigs 1 to 5 inclusive are injected intraperitoneally with 1 cc. of Antityphoid Serum W which agglutinates 1: 10,000.

Apr. 10. Guinea Pig 5, weight 220 gm., is injected intravenously with 2 cc. of typhoid extract. No immediate symptoms.

Agglutination titer of this animal 1: 80++

1: 160+

Apr. 12. Guinea Pig 4, weight 190 gm., injected intravenously with 2 cc. of typhoid extract. Typical symptoms; falls to side; characteristic breathing; definite but not fatal shock. As well as control in 8 minutes. Control, weight 195 gm., shows no immediate symptoms.

Nos. 2 and 4 titrated on this day show agglutination up to 1: 160.

(*b*) *Experiment 2.*—Oct. 18, 1916. A series of guinea pigs, weight 220 to 255 gm., is intraperitoneally injected with 2 cc. of antityphoid rabbit serum with a titer of 1: 4,000.

Oct. 20. These guinea pigs show a titer of 1: 100 to 1: 200.

Oct. 23. Agglutinins in guinea pigs now about 1: 100.

Oct. 24. Titer diminishing to 1: 50 in most of them, others partially agglutinated 1: 100. On this day one guinea pig is injected intravenously with 1 cc. of typhoid extract. No sign of anaphylaxis. This animal, however, died over night, whereas the unsensitized control of the same weight remained alive.

Oct. 25. Typical shock obtained in two of the guinea pigs; no immediate symptoms in controls on injection of 1 cc. of extract.

It would not aid much in throwing light on the subject of our paper were we to multiply experiments like the foregoing. We may summarize by stating that, after injecting intraperitoneally antityphoid serum, and waiting a period of 3 to 5 days, at a time when agglutinins are still present to a considerable degree in the circulation of the guinea pigs, definite shock can be observed on injection of antigen. Earlier than this, however, in spite of a higher concentration of antibodies in the blood, no definite or severe acute shock could be elicited. It has seemed that to obtain very severe symptoms an interval of considerably longer than 1 day is necessary when an intraperitoneal first injection is practiced.

The following experiment, one of several similar ones, shows that when relatively large amounts of antigen are used the differences between normal and sensitized guinea pigs are essentially quantitative and not qualitative:

Experiment 3.—Jan. 16, 1917. Guinea Pigs 6 and 7 are intraperitoneally sensitized with 2 cc. of antityphoid serum.

Jan. 19. The following injections are made.

Guinea Pig 6, weight 170 gm., receives intravenously 5 cc. of typhoid extract. Immediate shock; death in 8 to 10 minutes. Control, weight 200 gm., receives 5.5 cc. of same antigen; quite sick, coughs, scratches nose, breathes irregularly. Gradual recovery.

Guinea Pig 7 receives 4 cc. of antigen. Immediate difficult breathing, coughing, very sick. Died after 3 to 4 hours. Control, same weight, receives 4 cc. of antigen. Difficult breathing. Same type of illness as No. 7 but less severe. Both controls dead next morning. No. 7 dies in afternoon.

Although it has been an irregular observation, the immediate development of anaphylactic symptoms in normal guinea pigs injected with large amounts of typhoid antigen has occurred with sufficient frequency to force itself upon our consideration. A typical instance of this is the following:

Experiment 4.—Feb. 20, 1917. A normal guinea pig, weight 175 gm., received 3 cc. of typhoid extract intravenously at 12 noon. Immediate coughing, respiratory distress, staggering. Temporary improvement; died at 4 p.m.

This observation we think has important theoretical bearing when correlated with later experiments on the isolated uterus. In this place we wish merely to record it for future reference. It is interesting to note in this connection that, although the sensitized animals show a much greater tendency to immediate reaction, unless the shock is fatal, they rapidly improve and approach the condition of slower intoxication, ordinarily found in the normal animals similarly treated. The following experiment illustrates these conditions:

Experiment 5.—Feb. 28, 1917. Guinea Pig 8, weight 155 gm., sensitized intraperitoneally with 2 cc. of antityphoid serum on Feb. 24, was injected intravenously with 2 cc. of typhoid extract. At the same time a control normal animal, weight 135 gm., was similarly injected. The sensitized animal showed immediate symptoms; coughing, difficult respiration. The control showed no immediate effects. After 10 minutes the sensitized animal had improved and both were alike and growing progressively sicker. Both died on the same afternoon.

Although the preceding experiments seem to furnish sufficient evidence that the dominating mechanism of acute shock in bacterial anaphylaxis is not one of intravascular antigen-antibody reaction, we nevertheless thought it wise to repeat the experiments of Kraus

and Amiradžibi who obtained shock, as stated before, when they injected simultaneously bacteria and antiserum. This we did for the sake of completeness although we feel sure that Kraus and Amiradžibi's results were referable to the intrinsic toxicity of their rabbit serum for guinea pigs.

In the experiment which follows, one of several similar ones, antigen injections were made into guinea pigs passively sensitized, into normal animals, and into normal ones that had received intravenously 0.5 cc. of antiserum about an hour before inoculation with the antigen. One example of such an experiment follows.

Experiment 6.—Apr. 9, 1917. Guinea pig, weight 199 gm., passively sensitized. Agglutination titer on Mar. 30, 1:80. 1.45 p.m. Receives intravenously 2.5 cc. of typhoid antigen. Very sick immediately; breathes with difficulty and irregularly for 5 minutes then better. 9 p.m. Died.

10.45 a.m. Normal guinea pig, weight 200 gm., receives intravenously 0.5 cc. of typhoid antiserum. 1.55 p.m. Inoculated intravenously with 2.5 cc. of antigen. Not quite as sick as the preceding animal; illness develops more gradually but this animal does not recover from immediate symptoms as rapidly as the other. Dies next afternoon at 2.45 p.m.; *i.e.*, lives 6 hours longer than preceding guinea pig.

Normal guinea pig, weight 195 gm., inoculated with 2.5 cc. of antigen. 2.30 p.m. Very slightly sick, almost not at all; gradua ly sick. Very sick in p.m., but recovers and remains alive.

It would be of relatively little value for the purposes of this paper were we to protocol other experiments similar to or representing variations of the preceding. The results were by no means regular but, taken together, they pointed to certain fundamental conditions which are of considerable interest in connection with our later work.

We may summarize these facts by saying that: (1) Acute shock with convulsions and death is obtained more frequently and with lower dosage in sensitized than in normal guinea pigs. (2) Acute shock with death cannot be obtained with regularity in sensitized animals and is obtained in normal animals only on rare occasions when large doses are given. (3) To elicit acute shock with death even in sensitized animals requires at least a minimal fatal dose of the antigen and usually considerably more is required (two to three times) than the eventual killing dose of the antigen used. (4) Normal guinea pigs that are injected with a moderate dose of anti-

serum, followed within an hour by an injection of antigen, do not differ markedly from normal animals receiving the same amounts of antigen, in regard to acute symptoms. They have occasionally seemed sicker than the normal ones, but not with sufficient regularity to throw any light on any intravascular anaphylactic mechanism.

Analysis of Bacterial Anaphylaxis in Guinea Pigs.

We are confronted, therefore, with a problem which cannot be solved entirely by experiments on the living animal. On the one hand, our inability to obtain marked sensitiveness to relatively small doses, unless we allowed a considerable period to elapse between the administration of antiserum and the injection of antigen, points, as in serum anaphylaxis, to the importance of a cellular mechanism. On the other hand, the fact that with larger doses the differences between the sensitized and the normal become less well defined, though still apparent, would indicate that a preparation of the cellular elements by antibody absorption is not absolutely essential.

The alternatives are that either we are dealing with two reactions, one a purely intravascular one which is sufficiently powerful to cause acute shock with death only when very large amounts of antigen are used, and a cellular one which is in evidence only in sensitized animals, or that both in normal and in sensitized animals the mechanism is purely cellular and that the normal tissues of the guinea pig possess originally a slight degree of sensitiveness, analogous to normal antibodies in the circulation, which is merely increased by specific active or passive sensitization.

This problem can be further approached only by recourse to some of the physiological methods, such as heart and lung perfusion lately again applied to serum anaphylaxis by Manwaring, or the study of the isolated uterus—a method which has brought brilliant results in serum anaphylaxis in the hands of Dale[19] and Weil[20] and has lately been applied to the study of bacterial antibodies by Weil and

[19] Dale, H. H., *J. Pharm. and Exp. Therap.*, 1912–13, iv, 517.

[20] Weil, R., *J. Med. Research*, 1912–13, xxvii. 497; 1914, xxx, 317, 331; 1915, xxxii, 107.

Torrey[21] and by Manwaring and Kusama.[22] We accordingly have worked with all these methods but wish to report in this paper only on results obtained with the Dale method.

The first step in this work was to obtain typhoid anaphylactic reactions with the isolated uteri of animals intraperitoneally injected with antityphoid serum. Here again, we found that the interval after injection was much longer than we expected and uteri from these guinea pigs would often react only after 3, 4, and 5 days, several of those tested after 1, 2, and 3 days failing to react. The highest degree of sensitiveness is not acquired after injections of 2 cc. of serum (titer from 1:4,000 to 1:10,000) until about the 5th to the 8th day after injections, a time at which the agglutinins in the circulation of the animal are beginning to diminish. This point of the relation between the circulating agglutinins and the sensitiveness of the whole animal is one of great importance which we believe marks an essential difference between the study of the isolated uterus and the whole animal.

Fig. 1 represents the reaction of a passively sensitized uterus to typhoid antigen when the typhoid bacilli were ground in salt and were extracted with very slightly alkaline salt solution. The bath capacity in these earlier experiments was 200 cc.; in later ones this was reduced to 50 cc. for purposes of economy.

Fig. 2 represents a similar reaction of a highly sensitized uterus where it is shown that the sudden instillation of large amounts of typhoid antigen may give rise to a repetition of spasm, three or four separate shocks being noted. This has not often been the case in our experiments, the uterus usually going into a prolonged single spasm, but was interesting to us because it was a graphic representation of a repetition of shock which we once noticed in young goats on the reinjection of typhoid bacilli. It is also worth noticing that occasionally when a uterus has begun to relax, if more antigen is added, another shock can often be induced even though the first instillation seems to have contained much more antigen than could possibly be absorbed within the uterine cells, a fact which points to the importance of concentration rather than actual amount,

[21] Weil, R., and Torrey, J. C., *J. Exp. Med.*, 1916, xxiii, 1.

[22] Manwaring, W. H., and Kusama, Y., *J. Immunol.*, 1917, ii, 157.

since it is analogous to observations made by pharmacologists in similar experiments with drugs in which the essential element of action seems to depend upon concentration and not upon actual quantity. Repeated addition of the antigen, however, leads to rapidly diminishing intensity of shock and desensitization. Fig. 2 also incidentally seems to show that even when the surrounding antigen is removed, the stimuli which lead to spasm may, on occasion, continue, pointing to the essentially intracellular occurrence of the reaction between antigen and antibody which is the ultimate cause. We should emphasize, however, that the above is the only instance in which such repeated and strong reactions were obtained in this way.

Having thus ascertained that passive sensitization of the animal is easily determined by the Dale reaction, we thought that it would be easy by the same method to determine whether the shock elicited in normal animals by large doses of antigen depended merely upon a lesser degree of cellular sensitization. Were this the case, large amounts of antigen added to a bath containing a normal uterus should induce at least some degree of reaction. Figs. 3 and 4 represent these experiments.

It is apparent from both these charts that large amounts of antigen exert no effect upon the isolated normal uterus. From this it would seem clear that, whatever is the mechanism of injury which induces response of the whole animal to injections of typhoid antigen, it is not one in which cellular sensitiveness is involved, at least, in an anaphylactic sense. This observation we consider of great importance, for it indicates that to the mechanism of bacterial injury in the normal animal, there is superadded another and cellular one in the sensitized animal. It might, of course, be considered that cells other than uterine might be sensitive to direct reaction with the bacterial products in the normal guinea pig. But Weil, especially, has shown a remarkable parallelism between hypersusceptibility in general and uterine reactions in guinea pigs, and in the light of his work and that of others it would seem most likely that the normal animal lacks cellular sensitiveness to the unchanged bacterial substance and that the mechanism of injury, therefore, must be regarded as in large part an intravascular one until the animal is sensitized.

Incidentally, we have shown that with bacterial anaphylaxis the same phenomenon is present that was found in Dale's experiments with horse serum anaphylaxis; namely, that the normal uterus cannot be sensitized, even by prolonged soaking in immune serum, and the union with the antibodies in serum is one in which the antibodies become an intrinsic part of the cells and are not merely physically fixed to the cell exterior.

Up to this point we have worked with bacterial extracts and have found that, in fundamental principles, reactions obtained with bacterial antigen are essentially identical with those obtained with such antigens as horse serum, etc. Inasmuch as the bacteria in infectious diseases are present in the body primarily as living and growing cells, it would seem important to ascertain whether sensitive cells can react to the whole bacterial body, or whether, as we assumed above, there must be a preliminary extraction or solution of the bacteria before injury can be done to the tissue elements. In consequence, we carried out a number of experiments in which heavy suspensions of living bacilli were added to Locke's solution in which was suspended a uterus from a sensitized animal. The opposite horn was, of course, always tested for sensitiveness with typhoid extract. Figs. 5 and 6 illustrate these experiments and show that the whole bacilli exert little or no effect on the sensitized organ.

We must assume, therefore, that where bacteria are injected into the sensitized animal, or are invading the living animal or human body, injury to sensitized tissues must be preceded by a liberation of antigens. Does this occur in the blood stream by lysis? One way of determining this would be to add the serum of a guinea pig injected with whole typhoid bacilli some time before, into a bath containing a sensitized uterus. We tried this, but obtained confusing results owing to the fact that normal guinea pig serum in itself exerts irritating effects upon the isolated guinea pig uterus. In consequence, we resorted to precipitin and complement fixation reactions. The former were not satisfactory, but the latter gave us, we believe, a clear indication of the presence of dissolved antigen in the circulation of guinea pigs intravenously injected an hour previously with whole typhoid bacilli. Such an experiment follows.

Experiment 7.—Guinea pig, weight 300 gm., received five slants of living whole bacilli, washed once in salt solution, intravenously. After 1 hour the animal was bled and cultures were taken on agar plates from its whole blood and from the serum after clotting of the blood. The following experiment was then done with the inactivated serum of this animal as antigen; a strong antiserum as antibody; and a fresh guinea pig complement previously titrated carefully against the hemolytic system.

Tube.	Two units of complement added to the following.	Inhibition of hemolysis.
1	0.1 cc. of typhoid antiserum +1.5 cc. of salt solution	0
2	0.3 " " serum of injected guinea pig +1.2 cc. of salt solution	0
3	0.1 " " typhoid antiserum +0.1 cc. of serum of injected guinea pig +1.4 cc. of salt solution	+++
4	0.1 cc. of typhoid antiserum +0.2 cc. of serum of injected guinea pig +1.3 cc. of salt solution	++++
5	0.1 cc. of typhoid antiserum +0.3 cc. of serum of injected guinea pig +1.4 cc. of salt solution	++++
6	1.6 cc. of salt solution	0

To these tubes we added two units of complement and, after 1 hour in the water bath, sensitized red cells were added. The results obtained are expressed in terms of Wassermann reactions as read in 1 hour in the water bath and over night in the ice chest, this being necessary because 0.3 cc. of the injected guinea pig serum was slightly anticomplementary and absolutely complete reactions could not be read until the next morning in this tube and in Tube 5.

There is one possible error which, however, we think we can discount; namely, the culture taken from the whole guinea pig blood in this and other experiments always yielded considerable numbers of colonies of typhoid bacilli, as many as 1,000 or so, which inclines one to consider the possibility that the complement fixation might have been due to whole bacilli. However, the serum from such blood was entirely clear and was further centrifugalized before use, and cultures taken from the serum in this experiment before it was heated for inactivation yielded only two to three colonies per 0.2 cc., which indicated to us that, as one would expect, the great bulk of typhoid bacilli were caught and held in the clot. Had the clot been allowed to stand in the ice chest over night for the expression of the sera, it might still have been possible that an extraction of these typhoid bacilli might have taken place, but we centrifugalized the blood as

soon as clotted and extraction therefore seems unlikely. We believe, therefore, that these experiments indicate that in the circulation of the guinea pig injected with live typhoid bacilli, and presumably, therefore, in the spontaneously infected body, antigen is in some way liberated to the circulating blood, which then could react with sensitized tissue as in our uterus experiment.

If this is the case, the next link in our chain would be to determine whether we could also trace the actual formation of toxic products, such as Friedberger's anaphylatoxin, or Vaughan's split products, or the substances we have spoken of as proteotoxins in the circulating blood. We have attempted to do this in two ways. One consisted in repeated attempts to produce acute symptoms of shock in guinea pigs by injecting intravenously blood serum taken from normal animals rendered severely sick both by typhoid bacilli and typhoid extract. We have never succeeded in this. Next we attempted to produce contraction in the normal guinea pig uterus, by adding to the bath of Locke's solution such guinea pig serum. In this we did not succeed, partly because it was never possible to add large amounts of guinea pig serum to these baths without running the risk of obtaining the contraction incident to the instillation of normal guinea pig serum. When we did so, no marked contrast appeared between the result of putting in normal guinea pig serum and putting in the same amount of the serum of an animal dying of typhoid bacilli (Fig. 7).

We cannot, therefore, with any of the methods now at our disposal, demonstrate the formation in the blood stream of the typhoid-infected animal of the poisons which are responsible for acute anaphylactic shock. Does this negative the intravascular production of such toxic substances? We think not.

In the first place, it must be remembered that, whatever may be the mechanism of injury going on in an animal infected and poisoned with typhoid or other bacilli, the process is a gradual one extending over a period of from 4 to 12 hours or longer, even when large doses are given. It is likely that the poisons which are formed are absorbed with considerable speed, this being responsible for the symptoms developing in the animal. It is not likely, therefore, that we could find at any one time a sufficient amount of the toxic substance present in the limited quantity of blood serum which we can readily inject into

an animal or instil into the Dale's bath, to expect to obtain acute reactions. For with bacterial anaphylaxis especially, it must be remembered that acute shock is obtained with no great regularity and only in animals highly sensitized when considerable quantities of bacterial antigen are administered.

Figs. 8 and 9 demonstrate incidentally that the conditions observed with bacterial antigens are to a great extent similar to those observed when other cellular antigens, such as erythrocytes, are used. Fig. 8 shows a typical reaction with the uterus of a guinea pig which had received 2 cc. of amboceptor intraperitoneally 3 days before, when hemolyzed red cells were added to the bath, and also indicates subsequent desensitization; and Fig. 9 shows the complete failure of reaction when the cells are added as "whole" cells unhemolyzed to a similarly sensitized organ. Reaction can be elicited by cells hemolyzed with distilled water or hemolyzed directly in the Locke's bath by the addition of amboceptor and complement, thus showing the preliminary function of solution of the cells in the blood stream carried out by the circulating antibodies. We do not insert a chart to show this because these records are very long owing to the necessity of allowing plenty of time between the instillation of amboceptor, complement, and red cells, to eliminate non-specific irritation of the uterus by these substances separately. The results were clear-cut, contraction usually beginning at about the time when hemolysis began and progressively increasing towards its completion.

Another point which formed an important part of our scheme of work was the study of the possible influence of circulating antibodies upon the reaction between antigen and sensitized tissues. To obtain light on this subject, we proceeded by the following two methods. In one series of experiments antiserum was injected into sensitized animals just before the injection of antigen, in order to ascertain whether by this means shock was diminished or eliminated. In another series, antigen was incubated for from 1 to 3 hours with antiserum and the mixture instilled into Locke's baths containing sensitized uteri.

An experiment by the former manner of procedure is as follows:

Experiment 8.—Apr. 7, 1917. 2.30 p.m. Passively sensitized guinea pig, weight 190 gm., receives intravenously 0.5 cc. of strong typhoid antiserum. After 14 minutes receives 3 cc. of typhoid antigen. Very slight symptoms; breathing slightly labored but runs about immediately and shows hardly any illness for the first 10 or 15 minutes.

Passively sensitized guinea pig of the same lot as the preceding animal. Agglutinin titer about 1:160. Receives 3 cc. of typhoid antigen. Very sick immediately; coughs and breathes irregularly. Much sicker than the preceding guinea pig.

These two animals represent a few of a considerable number similarly treated. With a few exceptions it seemed fairly definite that the presence of a considerable concentration of antibodies in the circulation delayed rather than hastened the development of acute symptoms.

The explanation for this we think is to be found more definitely in the following experiments with isolated uteri.

Fig. 10 represents an experiment in which the typhoid antigen which in substance consisted of a turbid extract containing not only extracted dissolved substances, but also particles, was exposed to the action of antiserum until a heavy precipitate had formed. This precipitate was then washed and added to the Locke's bath. No reaction resulted. The clear supernatant fluid, however, gave a sharp reaction in all cases, whether or not we added an excess of antiserum and reincubated.

Inasmuch as our antiserum gave only a slight precipitation with clear, filtered typhoid extract, it remained uncertain whether or not the experiment represented by Fig. 10 meant only that undissolved particles had been precipitated or whether it also signified that a part of the dissolved antigen was rendered useless by the antibodies. After several unsuccessful attempts to obtain a clear answer to this query by working with typhoid antigen and antibody, we decided to repeat the experiment using human serum as antigen and antihuman rabbit serum as antibody. Fig. 11 represents such an experiment.

Here 0.5 cc. of human serum in 1 cc. of salt solution was incubated with 0.3 cc. of a strong antihuman rabbit serum. A heavy precipitate formed which was thrown down in the centrifuge and washed once with salt solution, then shaken up in a small amount of salt solution and added to the bath containing a passively sensitized uterus. No

TABLE I.

Date.	Antigen.	Normal guinea pigs.		Sensitized guinea pigs.	
		Weight.	Remarks.	Weight.	Remarks.
1916	*cc.*	*gm.*		*gm.*	
Mar. 31	3	240	Lives.	250	Sensitized Mar. 27. No immediate symptoms; dead next day.
May 5	2	227	Dead next a.m.	215	Immediate.
		205	Dead 5 to 6 hrs.	215	Sensitized Mar. 31. Symptoms marked; dead next a.m.
Apr. 13	2	195	Sick but no shock; died next a.m.	190	Sensitized on Apr. 8. Definite shock; dead same afternoon.
	1	180	No symptoms; lives.	180	Mild shock; dead same afternoon. Agglutinations of guinea pigs of same series up to 1 : 160.
Apr. 14	3 (ureter).	180	Immediate shock-like symptoms; dead next a.m.	180	Sensitized Apr. 8. Typical shock; dead next a.m. Sensitized sick more rapidly; mate agglutinin, 1 : 320.
Apr. 15	3	190	No shock; dead next a.m.	180	No shock; lives. Mate agglutinin, up to 1 : 80.
Oct. 24	1 (strong).	220	Slight symptoms; lives.	220	Sensitized Oct. 28. No shock; dead next a.m. Mate agglutinin, up to 1: 100.
1917					
Jan. 19	4	*	Immediately sick; died next a.m.	*	Sensitized Jan. 1. Heavier than control. Shock; died in 4 hrs. Mate agglutinin, 1 : 100.
	5	200	Distinct moderate shock; died next a.m.	170	5 cc. Acute shock; died.
Apr. 4	25	300	No symptoms; died next a.m.	270	Severe shock; lives. Agglutinin, 1 : 80.

*Error in records; no note of weights made.

reaction occurred. However, again the addition of the supernatant fluid to the same bath resulted in a strong reaction.

This experiment was in entire harmony with those performed with typhoid antigen and antibody. It showed that antigen precipitated by antibody was thereby neutralized as far as its powers to react with sensitized tissues were concerned. It also showed, incidentally, a fact immunologists have known for some time, that it is extremely difficult to remove all antigen from solution by precipitation with antibody, and that most of the substance of a specific precipitate is derived from the antiserum.

We still wished to determine if possible, whether an animal sensitized to typhoid protein was or was not thereby rendered more vulnerable; *i.e.*, more easily killed by typhoid antigen. Table I, constructed from scattered observations made from time to time throughout our work gives some clue to the question, though it by no means settles the matter conclusively.

It will be seen that as a rule the sensitized animals died more quickly that did the normal controls, in spite of the fact that they were slightly protected by the presence of small amounts of antibody. This point will need further experimentation, which is already in progress.

SUMMARY.

We have attempted in the preceding experiments the beginning of an analysis of bacterial anaphylaxis and its relation to the occurrences in the animal body during an infectious disease. We have shown that the sensitization of the tissues of guinea pigs, as indicated by the isolated uterus, required 3 to 5 days even when passive sensitization was employed, and that in these relations conditions with bacterial sensitization were entirely analogous to those revealed for serum anaphylaxis by Dale and Weil especially. It has become apparent that the sensitized uterus reacted not at all with whole bacteria or whole red cells, or, in other words, that before reaction with sensitized organs could occur an extraction or solution of the bacterial cell must take place. That bacteria yield some of their substance to the circulating blood during the course of infection was to be expected, but it has been definitely indicated, we think, by our complement fixations.

The mechanism of injury in the sensitized animal or in the human being so far along in typhoid fever that antibodies have begun to develop is in part one in which antigen, derived from the bacilli and brought into solution, or rather suspension, in the blood stream, reacts with antibodies which are from the beginning, or have subsequently become, integral parts of the cell protoplasm, the entire process taking place within the cell. This last point is indicated by the failure to sensitize by simply soaking the normal uterus in antiserum.

This, however, cannot be the entire story of injury. We know that typhoid antigen injected into normal animals in moderate amounts will render them gradually sick and eventually kill them. Also, a sufficient amount injected into a normal animal will occasionally produce acute symptoms, in every respect similar to the reaction produced in sensitized animals by smaller doses. We have shown that such acute symptoms in normal animals were not due in any degree to tissue sensitiveness, since even very large quantities of antigen will produce no response on the part of the normal uterus. It is reasonable to suppose, therefore, that the injury, gradual or acute, in the normal animal, is in no respect referable to tissue sensitiveness to the whole antigen, but rather must be referred to some series of phenomena which occur in the circulation. The acute shock of normal animals may possibly, therefore, be entirely due to an intravascular reaction. Whether this is one of antigen-splitting, or of antienzyme removal in the sense of Jobling is a point on which these experiments throw no light.

It is true that we have never succeeded in producing acute toxic symptoms either in the whole animal or in the isolated uterus with serum from animals acutely ill. This we eliminate as negative evidence inasmuch as we believe that the toxic substances need at no given time be present in the blood stream in sufficient concentration to render such an experiment successful. They are probably absorbed and do their injury almost as rapidly as formed, an assumption which is based on the speed with which symptoms develop.

It is possible, and not to be denied on the basis of any experiment that we can devise at present, that the gradual illness of the normal animal and the occasional acute shock of these animals may be based on entirely different mechanisms. In both cases, however,

in normal animals, they seem to be intravascular. And since the symptoms of acute shock which can be produced in sensitized animals with moderate doses can also, though only occasionally, be produced in normal animals with larger doses, it is reasonable to suppose that the poisons produced intracellularly in the one may be similar to those produced intravascularly in the other.

It does not seem likely that the specific circulating antibodies are in any way sources of increased injury to an animal spontaneously infected with bacteria. If sufficiently powerful at the beginning they may even prevent tissue injury, first by increasing phagocytosis, then by producing intravascular agglutination, and finally, as indicated by our experiments, even by removing a part of the antigen from possible reaction with the cell, though in this last respect our experiments indicate that they functionate imperfectly. It is more probable that their chief protective action to the sensitized body lies in removing the whole bacteria from the possibility of intravascular disintegration, which, as we have shown, is prerequisite to anaphylactic injury of the tissues of the host.

We would tentatively summarize our opinion as to the occurrences in the typhoid-infected body as follows: Early injury is probably due to disintegration of part of the bacteria in the course of which albumose-like bodies are liberated, and, following which, intravascular reactions result in the formation of toxic substances, perhaps by some form of proteolysis.

Since the accumulation of bacteria during these stages is relatively slight, this form of injury probably plays little part in producing symptoms. Indeed, the experiment by which acute injury is produced in the normal guinea pig by the sudden injection of several times the lethal dose of partly dissolved bacteria, finds no analogy in the spontaneously diseased body.

At this time the tissues are not sensitive, but as antigen absorption progresses and the tissues are stimulated to react, sensitiveness develops, which renders them much more delicately amenable to injury by direct reaction with even small amounts of dissolved but otherwise unaltered antigen. This process is directly counteracted by circulating antibodies which tend to remove the bacteria from the possibility of yielding their antigen to solution by agglutinating

them, aiding phagocytosis, and to a slight extent even neutralizing dissolved antigen.

It seems likely, therefore, that the symptoms which appear as the incubation time ends are largely those due to cellular sensitization which probably begins before any considerable amount of circulatory antibodies is present. The circulating antibodies would seem to have little or nothing to do with intravascular injury, the ferments responsible for this, however much it may occur, probably consisting of the non-specific proteases studied in this connection by Jobling.

Finally it appears that highly sensitized animals are more easily killed by typhoid antigen than are normal animals, provided they do not dispose over unusually large amounts of circulating antibodies.

Cure would consist of a gradual checking of growth and final destruction of the bacteria, and the consequent cessation of antigen liberation, but delicate hypersusceptibility would probably persist for some time after cure and immunity have been established. Just what the relation between tissue hypersusceptibility and immunity is remains a problem for further study.

EXPLANATION OF PLATES.

PLATE 38.

FIG. 1. Typical reaction of sensitized uterus to typhoid antigen.

FIG. 2. Repeated spasm on inoculation of large amounts of antigen. A reaction like this was obtained very exceptionally. No other was obtained in which the same degree of repetition was noticed.

FIG. 3. Failure of normal uterus to react to typhoid antigen. This uterus had been soaked in immune serum for 3½ hours.

FIG. 4. Failure of normal uterus to react to typhoid extract in spite of the repeated inoculation of large amounts. The amounts instilled in the bath in this case were at least four times greater than sufficient to kill a guinea pig of the same size.

FIG. 5. Failure of sensitized uterus to react to large doses of living typhoid bacilli.

PLATE 39.

FIG. 6. Another instance of the failure of living typhoid bacilli in large amounts to stimulate sensitized uterus.

FIG. 7. Failure of normal uterus to react when the serum of a guinea pig dying of living typhoid bacilli was added. Attempt to demonstrate proteotoxin in serum of a typhoid-infected animal.

FIG. 8. Typical reaction of the uterus of a guinea pig passively sensitized with amboceptor when hemolyzed red cells are added.

FIG. 9. Uterus of a guinea pig passively sensitized to red cells. The chart shows the failure of reaction when cells are intact and reaction when cells are disintegrated by hemolysis.

FIG. 10. Uterus passively sensitized to typhoid antigen. The chart shows the failure to react when precipitate produced by the action of antiserum is added. Reaction when supernatant fluid of such a mixture is added.

FIG. 11. Uterus passively sensitized to human serum. Failure of reaction when precipitate produced by reaction of human serum and antihuman serum is added, but prompt reaction when the supernatant fluid of such a precipitin reaction is instilled into the bath.

FIG. 1.

FIG. 2.

FIG. 5.

FIG. 4.

FIG. 3.

(Zinsser and Parker: Bacterial anaphylaxis and infection.)

FIG. 7.

FIG. 9.

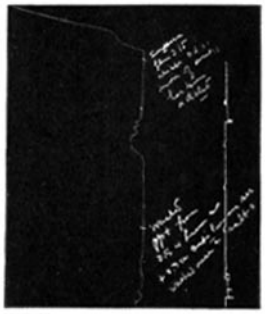

FIG. 11.

FIG. 6.

FIG. 8.

FIG. 10.

(Zinsser and Parker: Bacterial anaphylaxis and infection.)