

AN EXPERIMENTAL STUDY OF METHODS OF PRO-
PHYLACTIC IMMUNIZATION AGAINST
TYPHOID FEVER

*STUDIES IN TYPHOID IMMUNIZATION. V**

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INTRODUCTION

Beumer and Peiper¹ were undoubtedly the first fully to appreciate the possibility of an active immunization against infection with the typhoid bacillus. In 1887 they were able to prove that mice that have recovered from a non-fatal infection with living typhoid bacilli are frequently protected against subsequent, larger, and usually fatal doses of the same organism. In their most successful experiment they found that the best results were obtained by the gradual increase in dosage on successive inoculations, and they further suggest that it may be possible to immunize by means of sterilized cultures, which, as had already been shown, contain the toxic principle of the typhoid bacillus. They raise the question as to whether it might not be possible to immunize human beings by means of gradually increasing amounts of such killed cultures. In the following year Chantemesse and Widal,² following the work of Salmon and Smith³ on hog cholera, and of Roux and Chamberlain⁴ on malignant edema, found that they could protect mice against infection with living typhoid bacilli by means of sterilized cultures of the organism.

The practical application of these experimental results in animals to the prevention of typhoid fever in human beings did not come until eight years later, following the discovery of the lysins by Pfeiffer. It was A. E. Wright,⁵ who, in a preliminary publication in 1896 fol-

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1. Beumer and Peiper: Bakteriologische Studien über die ätiologische Bedeutung der Typhusbazillen, *Ztschr. f. Hyg.*, 1887, ii, 110.

2. Chantemesse and Widal: De l'immunité contre le virus de la fièvre typhoïde, conférée par des substances solubles, *Ann. de l'Inst. Pasteur*, 1888, ii, 54.

3. Salmon and Smith: On a New Method of Producing Immunity from Contagious Diseases, *Proc. Biol. Soc. Washington*, 1884-1886, iii, 29; *Centralbl. f. Bakteriol., Orig.*, 1887, ii, 543.

4. Roux and Chamberlain: Immunité contre le septicémie conférée par des substances solubles, *Ann. de l'Inst. Pasteur*, 1887, i, 561.

5. Wright: On the Association of Serous Hemorrhages with Conditions of Defective Blood-Coagulability, *Lancet*, London, Sept. 19, 1906, ii, p. 802.

lowed by a fuller account by Wright and Semple⁶ in the beginning of 1897, first outlined a method of immunizing human beings against typhoid fever. The method as outlined is notable on account not only of the essential facts involved, but also of the orderly and systematic method by which the problem was approached. Wright grew the culture of the typhoid bacillus in bouillon for two or three weeks and then killed them by heating to 63 C. (145.4 F.) for an hour, and preserved them with 0.5 per cent. phenol (carbolic acid). These vaccines were then tested for sterility and their toxicity carefully standardized by determining the minimal lethal dose for guinea-pigs; the dose chosen for injection in human beings was measured by this toxicity for animals. Wright further introduced a method of counting the number of the bacteria in the preparation employed by comparing their number in a given dilution when mixed with a suspension of red blood-cells, the number of which could be accurately determined. The dose of bacteria usually employed was from 750 to 1,000 million.

In the same year (1896) Pfeiffer and Kolle⁷ described their method of immunizing and their method of estimating the protection that was produced in human beings by the agglutinins and the bactericidal potency of the serum. They employed agar-cultures of an avirulent strain of the typhoid bacillus suspended in salt solution and killed by heating to 56 C. (132.8 F.). An amount of this suspension, corresponding to one-tenth of an agar culture of something like 2 mg., was usually given on the initial injection, which was, as a rule, the only one. It is frankly admitted that the symptoms produced by this amount of culture were severe, and the method has since been modified in several ways to avoid these symptoms without essentially changing the principle involved.

So much for the first two communications on typhoid immunization in human beings. They form the groundwork on which subsequent methods of vaccination against typhoid fever have been built. The various methods that have since been advocated are numerous. Metchnikoff and Besredka⁸ estimate that at least twenty different methods of vaccination have been described and advocated. Friedberger,⁹ in his systematic review on typhoid immunization, enumer-

6. Wright and Semple: Remarks on Vaccination Against Typhoid Fever, Brit. Med. Jour., Jan. 30, 1897, i, p. 256.

7. Pfeiffer and Kolle: Experimentelle Untersuchungen zur Frage des Schutzimpfung des Menschen gegen Typhus abdominalis, Deutsch. med. Wchnschr., 1896, xxii, 735.

8. Metchnikoff and Besredka: Recherches sur la fièvre typhoïde expérimentale, Ann. de l'Inst. Pasteur, 1911, xxv, 193.

9. Friedberger: Die Methoden der Schutzimpfung gegen Typhus, etc. Kraus and Levaditi: Handbuch der Technik und Methodik der Immunitätsforschung, Fischer, 1908, i, 722.

ates twelve recognized methods. Paladino Blandini¹⁰ has actually attempted to test the comparative immunizing value of seventeen preparations. It is not our purpose to describe all these methods in detail, and the reader who wishes further information on them may consult the systematic description of Friedberger⁹ or of Fornet¹¹ in regard to them. It will be well, however, to outline the most important of these methods as evidence of the scope that the investigation has taken in perfecting this type of immunization, and as indicating the tendency which would seem to be leading to its gradual perfection.

*PREPARATIONS OF THE TYPHOID BACILLUS THAT HAVE
BEEN USED AS VACCINES*

A. KILLED CULTURES OF THE TYPHOID BACILLUS

We have already mentioned that the first two preparations, those of Wright and of Pfeiffer and Kolle, consist essentially in killed cultures, the one, a bouillon-culture and the other a suspension of an agar-culture. These two original methods have been followed by many modifications. Thus Loeffler¹² took advantage of the fact that ferments, when dried, resist heating to a considerable degree without deterioration, and, regarding the antigenic property of the bacillus as ferment-like, dried suspended agar-cultures of the micro-organism and then heated them to from 120 to 150 C. (248 to 302 F.). These dried cultures were then pulverized and used in weighed amounts for immunizing animals. He states that such a culture has lost little of its property to produce antibodies. Friedberger and Moreschi¹³ use a similar dried and heated culture, and administer it intravenously in very small doses; for example, an amount corresponding to 1/4,000 oese in immunizing human beings. It should be noted at this point that the method currently employed of determining the immunizing value of these preparations lies in an estimation of the antibodies (agglutinins, lysins, etc.) produced. As we shall later have cause to consider, these estimations offer an indication rather of the reaction of the animal body than a sure means of determining the degree of protection that has actually been afforded.

As we shall see in a moment, the use of living instead of dead cultures has been warmly advocated by certain observers, and their

10. Blandini, Paladino: *Profilassi specifica del tifo addominale*, Ann. d'ig. sper., 1905, xv, 295.

11. Fornet: *Immunität bei Typhus*; Kolle and Wassermann: *Handbuch der pathologische Mikroorganismen*, Ed. 2, Fischer, 1912, iii, 837.

12. Loeffler: *Ueber ein neues Verfahren zur Gewinnung von Antikörpern*, Deutsch. med. Wchnschr., 1904, xxx, 1913.

13. Friedberger and Moreschi: *Vergleichende Untersuchungen über die aktive Immunisierung von Kannichen gegen Cholera und Typhus*, Centralbl. f. Bakteriol., Orig., 1905, xxxix, 453.

assertions have apparently convinced several who are not quite willing to adopt such preparations owing to their possible danger, although they endeavor to approach them as far as possible without actually using living micro-organisms. It is apparently now the consensus of opinion that bacterial cultures are most antigenic when employed as nearly as possible in their living, unaltered condition, and that heat in particular tends to alter or destroy essential, characteristic, antigenic properties. Several methods have been advocated for avoiding or obviating so far as possible the destructive influence of heat, and at the same time killing the bacteria. Leishmann¹⁴ advocates killing the typhoid bacillus at 53 C. (127.4 F.) instead of at 56 or 60 C. (132.8 or 140 F.). Vincent¹⁵ while fully recognizing the superior value of living cultures, regards their use as dangerous, and therefore kills the vaccines that he employs by means of ether. As will be later mentioned, we have used alcohol for the combined purposes of killing the typhoid bacilli and accelerating their flocculation and drying. Levy and Bruch¹⁶ killed their preparations by shaking the micro-organisms in a medium containing galactose, and find that organisms prepared in this way immunize guinea-pigs, as well as the living cultures, and that these two preparations are far superior to killed cultures in corresponding amounts. Fornet¹⁷ regards the unpleasant effects that are produced by the heated vaccine as due not only to the heating itself but also to the presence of a large amount of albumin in the culture-medium. He therefore grows his micro-organism in a medium containing only a small amount of peptone and kills them by heating to 55 C. (131 F.) for fifty-five minutes. Courmont and Rochaix¹⁷ killed their preparations by heating to 53 C. (127.4 F.), and further modified the usual method by administering the antigen through the rectum. Nicolle, Connor and Conseil¹⁸ heat their bacteria to 55 C. (131 F.) for forty-five minutes, and then to 52 C. (125.6 F.) for thirty minutes more, and inject intravenously. Wassermann¹⁹ insists that the antibodies produced by typhoid bacilli heated to 53 C.

14. Leishmann: Preliminary Note on Anti-Typhoid Vaccine in the Treatment of Enteric Fever, *Jour. Royal Army Med. Corps*, 1909, xii, 136.

15. Vincent: Sur la vaccination antityphique, *Jour. State Med.*, 1912, xx, 322; Sur l'immunisation active de l'homme contre la fièvre typhoïde, *Compt. rend. Acad. d. sc.*, 1912, clv, 480.

16. Levy and Bruch: Vergleichende experimentelle Untersuchungen zwischen drei Typhusvaccinen, die so wohl Bakterienleibersubstanzen als auch lösliche Stoffwechsel produkte enthalten, *Arbeit. a. d. k. Gsndtsamte*, 1913, xlv, 150.

17. Courmont and Rochaix: Immunisation antityphique de l'homme par voie intestinale, *Compt. rend. Acad. d. sc.*, 1912, cliv, 611, 1829.

18. Nicolle, Connor and Conseil: De l'inoculation intraveineuse des bacilles typhiques morts à l'homme, *Compt. rend. Acad. d. sc.*, 1912, clv, 1036.

19. Wassermann: Beiträge zur Typhus Schutzimpfung, *Ztschr. f. Hyg.*, 1911, lxx, 204.

(127.4 F.) are not markedly better than when they are heated to 56 C. (132.8 F.). Renaud²⁰ has advocated the use of ultraviolet rays to kill the bacteria.

B. EXTRACTS OF BACTERIA

In addition to killed cultures of bacteria, numerous extracts and preparations derived from the bacteria have been advocated for the purpose of immunizing against typhoid fever. Hahn²¹ has recommended the extract obtained from masses of bacteria by means of the Buchner press. McFadyen and Roland²² have utilized liquid air as a means of killing bacteria and obtained from them an extractive substance. Neisser and Shiga²³ have utilized free receptors obtained by autolysis of bacteria at body temperature in salt solution. Wassermann²⁴ has suggested a similar method with the autolysis produced by distilled water. He subsequently dries the extract obtained in this way and uses it as a vaccine powder (*Impfpulver*). Brieger and Mayer²⁵ have used a watery, filtered extract of shaken bacteria. Bergell and Meyer²⁶ have used an extract of dried bacteria obtained by treating them with dilute hydrochloric acid.

Various so-called soluble toxins of the typhoid bacillus have also been suggested for immunizing purposes by Chantemesse,²⁷ by Werner,²⁸ and by Rodet, LaGriffoul and Wahby.²⁹ The extract of bacteria obtained by the method of Jez³⁰ has also been suggested.

C. LIVING CULTURES OF THE TYPHOID BACILLUS

Living cultures of the typhoid bacillus, usually more or less modified in their pathogenicity, have been warmly advocated by certain

20. Renaud: Vaccinothérapie par les vaccins irradiés (Etude biologique du vaccin typhique), Presse méd., 1911, xix, 655.

21. Hahn: Immunisirungs und Heilversuche mit plasmatischen Zellsäften von Bakterien, München. med. Wchnschr., 1897, xlv, 1347.

22. McFadyen and Roland: On the Intracellular Constituents of the Typhoid Bacillus, Centralbl. f. Bakteriöl., Orig., 1903, xxxiv, 618.

23. Neisser and Shiga: Ueber freie Receptoren von Typhus und Dysenterie Bazillen und über Dysenterietoxin, Deutsch. med. Wchnschr., 1903, xxix, 61.

24. Wassermann: Zur aktiven Immunisierung des Menschen, Festschr. z. 60 Geburtst. v. Robert Koch, Fischer, Jena, 1904, p. 527.

25. Brieger and Mayer: Weitere Versuche zur Darstellung spezifischen Substanzen aus Bakterien, Deutsch. med. Wchnschr., 1903, xxix, 309.

26. Bergell and Meyer: Ueber eine neue Methode zur Herstellung von Bakterien Substanzen, welche zur Immunisierungszwecken geeignet sind, Med. Klin., 1906, xli, 753.

27. Chantemesse: Toxine typhoïde soluble et sérum antitoxique de le fièvre typhoïde, Progrès méd., 1898, iv, 16.

28. Werner: Sur la toxine excrétée par le bacille typhique, Compt. rend. Soc. de biol., 1904, lvi, 836.

29. Rodet, Le Griffoul and Wahby: La toxine soluble du bacille d'Eberth, Compt. rend. Soc. de biol., 1904, lvi, 794.

30. Jez: Ueber Typhus Behandlung (Abdominal Typhus), mit einen anti-typhus Extract, Wien. med. Wchnschr., 1899, xii, 346.

observers as producing the best immunizing preparations in a manner similar to the methods that have been employed in dealing with other diseases, notably in cholera (Strong and Kolle). Castellani³¹ uses an avirulent strain of the typhoid bacillus in the form of recent bouillon-cultures which are then partially killed by heating to 50 C. (122 F.) for one hour. Such a modified culture produces rather severe local and general symptoms, but when given twice would, to judge from Castellani's results, produce a most satisfactory degree of immunity, which apparently has lasted in a number of cases on which he reports for at least four years. He suggests, as an alternative, that the first injection may consist of a killed culture followed by a living culture on the second inoculation. In addition to the superior immunizing properties of the living culture, it is also pointed out by Fornet¹¹ that the killed cultures in a given dose give more reaction because the split products of proteins, which are recognized to be toxic, are liberated by heat. Living cultures have also been employed by Pescarolo and Quadroni.³² The form of living cultures which has been advocated by Besredka will be considered under the next heading. As has already been mentioned, living cultures are generally admitted to be of superior immunizing value by many who are not willing to adopt them, owing to the real or fancied dangers coincident with their use, and this has led to an attempt to approach the condition of living bacteria without actually employing them. (Compare Vincent,¹⁵ Levy and Bruch,¹⁶ and particularly Metchnikoff and Besredka, to be considered presently.)

D. SENSITIZED CULTURES OF THE TYPHOID BACILLUS

The method of active immunization by means of sensitized vaccines, that is to say, by cultures that have been first treated with an immune serum and then killed, was introduced by Besredka³³ in 1902. This method is not infrequently referred to as serovaccination, but it differs from the method properly called serovaccination suggested by Leclainche³⁴ in swine erysipelas and by Calmette and Salimbeni³⁵ in plague, in that the excess of immune serum which these authors used is removed from the treated bacteria. It was found, as Besredka notes, that this excess serum tends to produce simply a passive immunity instead of the active immunity which is produced by the cultures treated with immune serum, and washed. Apart from his

31. Castellani: Typhoid and Paratyphoid Vaccination with Live Attenuated Vaccines, *Lancet*, London, 1912, i, 583; Observation on Typhoid Vaccination in Man with Attenuated Living Cultures, *Centralbl. f. Bakteriol.*, 1909, lii, 92.

32. Pescarolo and Quadroni: Aktive Immunisation durch subcutane Injektion lebender Typhusbazillen bei Eberthschen Infection, *Centralbl. f. inn. Med.*, 1908, xxix, 989.

original experimental work, Besredka did not deal with the practical aspects of sensitized vaccines until the experimental work on typhoid fever in apes was taken up by him in collaboration with Metchnikoff in 1911.⁸ In the meantime, however, sensitized vaccines had been tried out apparently with considerable success in at least three instances. Marie³⁶ had been able to utilize the principle in treating rabies virus, Dopter³⁷ in vaccination against dysentery, and Theobald Smith³⁸ in a similar way found that he could produce active immunity by a balanced mixture of diphtheria toxin and antitoxin. This method of active immunization against diphtheria by a balanced (sensitized) mixture of toxin and antitoxin has recently been applied to human beings by von Behring.³⁹ The principal advantages of this method, as originally pointed out by Besredka and as apparently proved, are first, it produces little or no violent reaction on inoculation in instances in which the untreated bacteria themselves are distinctly irritating, as, for example, in plague. Secondly, it gives rise to an immediate though transitory passive immunity. Thirdly, it produces, eventually, an active immunity which is as enduring and as rapidly formed as when untreated bacteria are used.

Not a little experimental work was done with sensitized typhoid vaccine before the work of Metchnikoff and Besredka, which will be taken up later. Paladino Blandini¹⁰ made a very careful study of seventeen different typhoid vaccines, comparing their relative immunizing properties. His experiments were carried out on guinea-pigs, which were treated by the various preparations and subsequently given an intraperitoneal dose of living typhoid bacilli. The vaccines tested include small doses of living cultures; killed cultures prepared after the method of Pfeiffer and Kolle, and of Wright; several "soluble toxin preparations" of the typhoid bacillus; nucleo-albumins; and extracts of the typhoid bacillus prepared in different manners. Compared with these methods was the sensitized vaccine employed by

33. Besredka: De l'immunisation active contre la peste, le choléra et l'infection typhique, *Ann. de l'Inst. Pasteur*, 1902, xvi, 918.

34. Leclainche: Sur la sérothérapie du rouget du porc, *Compt. rend. Soc. de biol.*, 1897, xlix, 428.

35. Calmette and Salimbeni: La peste bubonique: Etude de l'épidémie d'Oporto en 1899, *Ann. de l'Inst. Pasteur*, 1899, xiii, 865.

36. Marie: Immunisation par des mélanges de virus rabique et de sérum antirabique, *Compt. rend. Soc. de biol.*, 1902, liv, 1364.

37. Dopter: Vaccination préventive contre le dysentérie bacillaire, *Ann. de l'Inst. Pasteur*, 1909, xxiii, 677.

38. Smith, Theobald: Active Immunity Produced by So-Called Balanced or Neutral Mixtures of Diphtheria Toxin and Antitoxin, *Jour. Exper. Med.*, 1909, xi, 241.

39. Von Behring: Ueber ein neues Diphtherie Schutzmittel, *Deutsch. med. Wchnschr.*, 1913, xxxix, 873.

Besredka, and Blandini was able to demonstrate that the latter method was by all means the most protective. Not only were guinea-pigs protected for at least four months, but it was found that their serum, which contains sensitizers, also protects normal animals against infection. Ardin-Del-teil, Negre, and Raynaud⁴⁰ find that the use of sensitized typhoid vaccine in rabbits and human beings gives rise to relatively small amounts of agglutinins, but the bactericidal properties of the serum of those treated in this manner are much higher than of those treated by the ordinary cultures. These authors have also obtained very favorable results in treating cases of typhoid fever with this vaccine.

This failure of sensitized or agglutinated cultures to produce potent antibodies had already been noted by Neisser and Lubowski⁴¹ and by Pfeiffer and Bessau.⁴² Garbat and Meyer⁴³ immunized rabbits either with sensitized or with whole cultures and compared the properties of the serums of the two sets. The serum obtained by immunizing the rabbits with sensitized cultures agglutinated and gave the fixation reaction much less strongly than the corresponding animals treated with unsensitized cultures. The serums of the sensitized animals, however, were more bacteriotropic and protected animals experimentally much better than the serum of the animals treated with the plain vaccine. This fact is incidentally evidence of the unreliability of the agglutinin test as a measure of the grade of protection against typhoid infection, a matter which we shall later consider.

In 1911 Metchnikoff and Besredka⁸ first reported their very important work on experimental typhoid fever in anthropoid apes. They found that the chimpanzee and the gibbon when given the dejecta from cases of typhoid fever, or lavishly contaminated food, or, in their latter experiments, when the mouth of the animal is painted with cultures of the typhoid bacillus, after eight days' incubation develop characteristic typhoid fever. Fifteen or sixteen monkeys that were tested gave positive blood-cultures on the tenth day, their serum contained agglutinins, and, in three instances, death due to the typhoid

40. Ardin-Del-teil, Negre and Raynaud: Sur la vaccinothérapie de la fièvre typhoïde, *Compt. rend. Acad. d. sc.*, 1912, clv, 1179; Recherches sur les réactions humorales des maladies atteints par la fièvre typhoïde traités par le vaccin de Besredka, *Compt. rend. Soc. de biol.*, 1913, lxxiv, 371; Recherches cliniques et expérimentales sur la vaccinothérapie de la fièvre typhoïde par le virus sensibilisé de Besredka, *Ann. de l'Inst. Pasteur*, 1913, xxvii, 644.

41. Neisser and Lubowski: Lässt sich durch Einspritzung von agglutinierten Typhusbazillen eine Agglutininproduktion hervorrufen? *Centralbl. f. Bakteriöl., Orig.*, 1901, xxx, 483.

42. Pfeiffer and Bessau: Zur Frage der Antiendotoxine bei Thyphus abdominalis, *Centralbl. f. Bakteriöl., Orig.*, 1910, lvi, 344.

43. Garbat and Meyer: Ueber Typhus Heilserum, *Ztschr. f. exper. Path. u. Therap.*, 1910, viii, 1.

infection followed. The temperature of these animals ran as high as 40.8 C. (105.4 F.). Peyer's patches were found swollen but not ulcerated. The only detail in which this experimental disease would seem to differ from typhoid fever in human beings is the fact that the spleen in the chimpanzees is not distinctly enlarged. Having determined in this way that the typhoid bacillus is in reality the cause of typhoid fever and not some adherent filterable virus, as in the case of hog cholera, they turned their attention to methods of immunization against the disease. Their first series of experiments are far from convincing, although they draw from them rather sweeping conclusions. In the first place, the number of animals employed was necessarily small owing to their expense and the great susceptibility of the animals to extraneous infection, so that in this first series, in which there were five experiments, there was only one vaccinated animal in each case, tested with an untreated control. They come to the conclusion from their preliminary attempts that neither Vincent's typhoid vaccine, an autolysate of the typhoid bacillus, nor a *killed* culture sensitized according to the method of Besredka, will immunize anthropoid apes against a subsequent infection by the mouth.

Apart from the necessarily small number of animals in each experiment, certain other objections may be made to their experiments and conclusions, part of which Vincent⁴⁴ has pointed out. In the first place, the animals were tested very shortly after finishing the immunizing treatment, usually in from four to six days, which may reasonably be regarded as too brief a period for the establishment of the highest grade of immunity; and, secondly, the dose given the animals by the mouth, which is not stated very definitely, seems in all events to be extremely large, much larger, indeed, than in the natural infection in man. They conclude from their experiments that heated vaccines do not protect anthropoid apes against typhoid infection, and, owing to the analogy of the disease in these animals with the human syndrome, they regard such vaccines as unfitted to protect human beings. Inasmuch as the protective vaccination of man against typhoid fever by means of heated vaccines is generally recognized as reducing the morbidity to one-half or one-sixth of the normal, the logic of their position does not seem sound. It would seem better to have concluded that the immunization of the disease in anthropoid apes, as they have produced it, is not similar to their analogs in human beings.

In their second communication, Metchnikoff and Besredka⁴⁵ present experiments which, with the exception of one experiment in

44. Vincent: Remarques sur la vaccination antityphique, *Ann. de l'Inst. Pasteur*, 1911, xxv, 455.

45. Metchnikoff and Besredka: Des vaccinations antityphiques, *Ann. de l'Inst. Pasteur*, 1911, xxv, 865.

which Vincent's vaccine is used, are more convincing. Their second series deals with the protective effect of vaccination by a living paratyphoid B culture, and by sensitized *living* cultures of the typhoid bacillus against subsequent infection of the latter organism in the manner described. These experiments comprise more animals than the previous series, and in addition the infection is not given until from ten to fifteen days after the completion of the treatment. Vincent's culture again failed to protect, whereas the living sensitized typhoid culture and the living paratyphoid B cultures uniformly saved the animals. It would be far from safe, however, to conclude from these experiments that no preparation of heated typhoid bacilli has the power to protect even anthropoid apes against subsequent infection by the typhoid bacillus.

We have examined the experimental details of the work of Metchnikoff and Besredka not in a spirit of unfavorable criticism, because, as will later be shown, we personally believe that their conclusions were in part correct, though the data on which they have based them have not convinced us.

We may here examine the results that have already been obtained in immunizing human being by the living sensitized cultures recommended by Metchnikoff and Besredka. Broughton Alcock,⁴⁶ working under their direction, was the first to report on the harmlessness of the method, its freedom from untoward effects and its apparent protective value. He advocates a dose corresponding to 1/100 of an agar-culture, which is equivalent to 500 million typhoid bacilli. Their organisms are sensitized by a few drops of a strong anti-typhoid serum, the exact potency and amount of which is not stated either by Metchnikoff and Besredka or by Broughton Alcock. After standing for twenty-four hours the clumped bacteria are washed in salt solution and resuspended in an aliquot portion of normal saline. A living sensitized vaccine prepared in this manner keeps for at least four months on ice. Objections have been raised by Vincent and others as to the danger of injecting living micro-organisms into the human body, but Metchnikoff and Besredka⁴⁷ have reported that careful tests fail to show that human beings treated in this manner become carriers of the typhoid bacillus or eliminate the micro-organism through the feces or urine. In view of the uniformly good results that have been obtained, there seems little reason further to suspect the harmfulness of the method. In a recent report Metchnikoff and

46. Alcock, Broughton: Vaccination for Typhoid by Living Sensibilized Typhoid Bacilli, *Lancet*, London, 1911, ii, 497.

47. Metchnikoff and Besredka: Sur la vaccination contre la fièvre typhoïde, *Compt. rend. Acad. d. sc.*, 1912, clv, 112.

Besredka⁴⁸ have further endeavored to determine the optimal vaccinating dose and interval of this sensitized vaccine, and Besredka⁴⁹ reports some of the favorable results that have been obtained. Cadeau⁵⁰ vaccinated twenty-five patients, and only two showed slight general symptoms. In an asylum at Briqueville where many cases of typhoid fever existed in 1912, 516 persons were vaccinated, and in the twelve months subsequent, no cases occurred among them whereas four occurred in the 343 persons who were not immunized. Marie reports similar absence of general reaction. In all, Besredka has dispensed 10,000 doses of the vaccine and no unpleasant results have been reported except when the injections were given intramuscularly. He recommends a dosage of from 500 million to 1,000 million, although larger amounts could be given without harm. It is, of course, too early to draw any conclusive figures as to the actual protective value of this method, although its harmlessness and freedom from untoward symptoms seem to have been proved.

PERSONAL INVESTIGATIONS

In view of the general acceptance of the relative protective value of immunization with killed cultures of the typhoid bacillus, and the claim that this method of vaccination with sensitized living typhoid bacilli insures all the advantages of the more unpleasant method of treating with dead cultures, and protects as well or better, it would seem desirable to devise some method of animal experimentation which would enable us to settle conclusively this and many other debatable questions that arise in connection with this extremely beneficial process. There are still many questions in connection with typhoid vaccination which remain unsettled, such as the dosage to be employed, the interval at which the injection should be given, and most important of all, the duration of the protection that is afforded by any particular method and to any particular individual. Animal experimentation has, to be sure, been used for the most part on guinea-pigs that after treatment with various typhoid vaccines are infected in the peritoneal cavity. It seems justifiable, however, to question the transference of results obtained in preventing a banal peritonitis in this animal to human beings. The method of testing typhoid vaccines on anthropoid apes that Metchnikoff and Besredka have described is undoubtedly the ideal one, but practically impossible to employ for the settling of details of method owing to the expense of the animals and the difficulty in obtaining them in any numbers.

48. Metchnikoff and Besredka: Des vaccinations antityphiques, *Ann. de l'Inst. Pasteur*, 1913, xxvii, 597.

49. Besredka: Deux ans de vaccination antityphiques avec du virus sensibilisé vivant, *Ann. de l'Inst. Pasteur*, 1913, xxvii, 607.

50. Cadeau: Cited by Besredka (49), *Ann. de l'Inst. Pasteur*, 1913, xxvii, 607.

We have already described in some detail our method for testing the immunizing power of a given typhoid vaccine by means of the "rabbit-carrier" condition.⁵¹ By employing one-half of a standard 10 per cent. blood-agar culture of a certain strain of *B. typhosus*, we have been able to produce this condition in normal rabbits with relative certainty. Thus of thirty control animals, twenty-eight, or 93 per cent., either became permanent carriers with positive cultures from blood or gall-bladder or, in a few instances, died acutely within twenty-four hours with symptoms of intoxication. For further discussion of the experimental advantages of this method over others proposed for this purpose, excepting experiments on anthropoid apes, we refer readers to our previous communication.

As we have just suggested, the presence of antibodies, particularly of agglutinins, is not a measure of the degree of protection against typhoid fever either in animals or in human beings. Antibodies indicate reaction which usually parallels protection but is by no means synonymous with it. In typhoid fever in particular the eventual absence of agglutinins in those recovered from and thereby protected from the disease indicates that serum tests are by no means to be confused with protection, which is more likely to be of a cellular than of a serum type. This indicates emphatically the necessity of testing the prophylactic immunizing value of any given vaccine by actual infection of the vaccinated animal rather than by any test of its antibodies. In our previous paper we have put forward our reasons for regarding the artificially produced bacteriemia in rabbits as analogous to typhoid fever in human beings, which with increasing agreement is now regarded primarily as a bacteriemia rather than a purely intestinal disease. The fact that we are able to prevent this carrier condition by previous immunization with one typhoid vaccine, whereas another preparation of the typhoid bacillus in the same amount does not prevent it, is further evidence that the method we have chosen is a delicate one and further suggests the variations in result that have been obtained in using different typhoid vaccines in human beings.

PREPARATION OF TYPHOID VACCINES FOR COMPARATIVE TESTS

The first essential in comparing various vaccine preparations seemed to us to lie in a standardization of the amount of substance employed. The methods usually employed in standardizing vaccines for clinical use may be accurate enough in view of the fact that the

51. Gay, F. P. and Claypole, E. J.: The "Typhoid Carrier" State in Rabbits as a Method of Determining the Comparative Immunizing Value of Preparations of the Typhoid Bacillus, *Studies in Typhoid Immunization I*, THE ARCHIVES INT. MED., 1913, xii, 613.

optimal dose is largely empirical and a little less or more may make no difference in the results. The fact that the usual methods of enumeration of bacteria by counting by the Wright method are being constantly changed is evidence enough that it is a far from satisfactory condition. We had already adopted the far more accurate method of dried, weighed bacterial preparations when the work of Wilson and Dickson⁵² came to our notice. These authors have carefully determined the actual weights of the doses of various bacteria as commonly employed, checking their weighing results by counting the bacteria in a given volume of suspension. They find, for example, that there are on an average 8,000 million of typhoid bacilli in 1 mg. of a typhoid vaccine dried on platinum-foil. We were surprised to find how closely our estimations, although obtained in a different manner, agree not only among themselves, but also with the results of these authors. Our method of preparing dead typhoid vaccine has been to add normal saline (0.85 per cent.) in proportionate amounts to suspend forty-eight-hour cultures grown on Blake bottles containing 125 c.c. of 2 per cent. agar (titrated + 1) or, in certain experiments, of the same medium containing 10 per cent. fresh defibrinated rabbit blood. To such a suspension is added an equal volume of absolute alcohol, which kills the culture in the proportions used in less than fifteen minutes, flocculates out the organisms so that they can be easily collected by centrifugalization, and accelerates their drying in an unglazed porcelain plate in partial vacuum over sulphuric acid. Estimations of the number of typhoid bacilli in two different suspensions by counting (Wright's method) or by plating in successive dilutions, and by weight are summarized in the following experiments:

EXPERIMENT 1.—Vaccine 8: Nine Blake bottles containing 10 per cent. rabbit blood-agar were inoculated with a blood bouillon culture of *B. typhosus* 3b and incubated for forty-eight hours. The combined growths were suspended in a total of 220 c.c. of normal saline and shaken; 20 c.c. were kept for plating; 200 c.c. were precipitated with 200 c.c. of absolute alcohol, centrifugalized and dried *in vacuo* over sulphuric acid. The dried residue was ground for one hour in a mechanical ore-grinder (in a wooden case) sterilized by formaldehyd fumes and containing an agate mortar and pestle run by a small motor. This residue of microscopically disintegrated bacterial bodies weighed 675 mg.

By successive plates the bacteria in 1 c.c. of the original suspension were estimated to be 25,500 millions. In 200 c.c. the bacteria, weighing 675 mg., should equal 5,100,000 millions, or 7,555 millions to the milligram.

EXPERIMENT 2.—Vaccine 9: Nine Blake bottles of plain agar were inoculated with a bouillon culture of *B. typhosus* 3 and incubated for forty-eight hours. This growth was suspended in 200 c.c. of saline and the subsequent treatment was the same as in Experiment 1, except that the numerical estimation of the

52. Wilson and Dickson: Rapid Gravimetric Method of Standardizing Vaccines, Jour. Hyg., 1912, xii, 49.

living bacteria was made by comparison with red blood-cells in a stained preparation (Wright's method).

The weight of dried ground culture equaled 360 mg. We draw attention again⁵¹ to the fact that the growth on blood-medium of *B. typhosus* is approximately twice that of the growth on agar. By count a cubic centimeter contained 13,000 millions, or 2,600,000 millions to 200 c.c., giving 7,222 millions to the cubic centimeter.

These two estimates of 7,555 millions and 7,222 millions of typhoid bacilli to the milligram may be compared with the 8,000 millions of Wilson and Dickson. Our interest in these figures, however, lies not in proving that the various methods of counting bacteria in our own and others' hands give surprisingly concordant results, but as giving us a comparison of weight to number so that one may state roughly in experimental and clinical work the weight dosage of the typhoid preparation required to give an amount comparable to, say, the classical 500 million bacteria ordinarily employed. There can be no question that in experimental work weighed amounts of dried preparations are more comparable and easier to determine than counted suspensions. Assuming the counts of Wilson and Dickson to be more accurate, the weighed amount of dried typhoid bacilli corresponding to a dosage of 500 million bacteria would be $\frac{1}{16}$ mg.

In all our experiments with vaccine preparations, then, we have used weighed amounts of dried and ground bacilli as a basis for comparison, except in those cases in which living cultures of the organisms have been employed, in which case comparisons were made in another way.

METHOD OF IMMUNIZATION

Having determined on the use of weighed amounts of dried, ground bacteria for comparative testing of typhoid vaccines, the next essential was to decide on the best method of immunization of the rabbits that are to be tested for protection against the carrier state.

Fornet and Müller⁵³ and Bonhoff and Tsuzuki⁵⁴ have already shown that for the production of certain antibodies an intensive method of immunization may be employed by reinjection at short intervals. One of us (Gay), in collaboration with Fitzgerald,⁵⁵ has still further perfected this method. We were able, for example, to produce high-grade hemolysins to sheep corpuscles by injecting rabbits intravenously on three successive days with 1 c.c. of washed

53. Fornet and Müller: Zur Herstellung und Verwendung praezipitierenden Sera, insbesondere für den Nachweis von Pferdefleisch, Ztschr. f. biol. Tech. u. Methodik, 1908, i, 201.

54. Bonhoff and Tsuzuki: Ueber die Schnellimmunisierungsmethode von Fornet und Müller, Ztschr. f. Immunitätsforschung, 1910, iv, 180.

55. Gay and Fitzgerald: An Improved Rapid Method of Producing Precipitins and Hemolysins, Univ. Cal. Pub. Path., 1912, ii, 77.

sheep blood. These lysins we find to be present in high degree four or five days after the last injection. Similar, although not quite so perfect, results were obtained in the production of precipitins and also in the production of bacterial agglutinins by Miss Edna Locke.⁵⁶ On the basis of these experiments, we had already determined that, provided a vaccine did not produce too severe symptoms, the period over which immunization extended might be markedly decreased without any corresponding deduction in the intensity of the immunity effected. It is of interest to note that Fornet¹¹ has also adopted a rapid method of immunization in vaccinating against typhoid. We have ourselves recommended, and Force⁵⁷ has employed immunization at intervals of two or three days in human beings, as will be mentioned later. The rapid method of immunization at short intervals not only may be shown in the case of rabbits to effect an equally high grade of immunity as determined either by antibody content or by actual resistance to infection, but also has the further advantage of actually producing less harmful effects when large amounts of the untreated vaccine are used. We have repeatedly noted that if a large total amount of vaccine is given to rabbits during a period extending over three weeks there is a larger percentage of mortality due to cachexia than when the same amount of vaccine is given within three days. The immunizing doses have invariably been three in number and have been administered either intravenously or subcutaneously; in all our later experiments it has seemed better to adopt the intravenous method as giving greater uniformity of results.

The advantage of immunization at short intervals as against longer intervals was first tested by comparing the resistance to the carrier state of two sets of rabbits, the one immunized by three intravenous injections on three successive days, the other by three equal doses administered intravenously at three-day intervals. There was found to be no marked difference in relative resistance of these two sets of animals when given the test dose two months later. The agglutinins averaged the same titer in the two sets. In a more systematic experiment, a comparison of three daily with three five-day interval doses was made (Experiment 3).

EXPERIMENT 3.—Fifteen rabbits weighing from 2,000 to 2,500 gm. each were divided into two lots of eight and seven animals and immunized as follows:

Lot 1: Eight animals were given three doses of $\frac{1}{6}$ mg. of dried, sensitized⁵⁸ Vaccine 13 intravenously in a volume of 1 c.c. 0.5. per cent. carbolated saline

56. Locke, Edna: A Rapid Method of Producing Bacterial Agglutinins, Univ. Cal. Pub. Path., 1912, ii, 91.

57. Force: Institutional Vaccination Against Typhoid Fever, Am. Jour. Pub. Health, 1913, iii, 750.

58. Gay, F. P., and Claypole, E. J.: The Typhoid "Carrier-State" in Rabbits

solution, Oct. 6, 13 and 22, 1913, that is, a total of 0.5 mg. in a period of sixteen days.

Lot 2: Seven animals were given three doses of $\frac{1}{6}$ mg. of dried, sensitized Vaccine 13 intravenously, Oct. 20, 21 and 22, in a volume of 1 c.c. 0.5 per cent. carbolated saline solution, that is, a total of 0.5 mg. in a period of three days.

Before test inoculation, in Lot 1, three animals died with extreme emaciation and no distinctive lesions (cachexia) — one of an intercurrent infection and one of injury. That is, in four of the eight animals death may reasonably be attributed to the vaccine. No animals in Lot 2 died during this period.

On the forty-fourth day after the end of immunization, the survivors of Lot 1, and those of Lot 2 were given each one-half standard blood-agar culture of *B. typhosus* 3b intravenously. Necropsies were performed on the animals that died, cultures were made from the blood and bile. The survivors were chloroformed, and cultures made from blood and bile on the ninth day. When organisms of proper morphology and cultural characteristics were isolated from either source they were tested by agglutination with a strong antiblood typhoid serum.⁵⁸ The results of this experiment may be summarized in Table 1.

TABLE 1.—RESULTS OF LONG AND SHORT-INTERVAL METHODS OF TYPHOID IMMUNIZATION IN RABBITS

	Died During Vaccination	Carriers	Recovered
Short intervals	0	3	4
Long intervals	4	3	0

This experiment as well as other similar ones would seem to indicate the superior value of the short-interval intensive method of immunization.

DOSAGE OF VACCINE PREPARATIONS

We have no contribution to make on the optimal dose of typhoid vaccine for prophylactic use in human beings. As will be seen later, our choice for such purposes has been largely empirical and has followed accepted usage. In our comparative experimental work, however, it has been necessary not only to use preparations derived from weighed, dried bacteria, but also to determine with increasing accuracy the duration of the protection afforded by a given amount of a standard preparation. In our first experiments we immunized separate series of rabbits each with a given preparation and then tested representatives of each series at several successive periods. Inasmuch as we started with rather large doses of vaccine, the time before the protection wore off was rather long and the method time-consuming.

as a Method of Determining the Comparative Immunizing Value of Preparations of the Typhoid Bacillus. Studies in Typhoid Immunization I. Agglutinability of Blood and Agar Strains of the Typhoid Bacillus, Studies in Typhoid Immunization II, THE ARCHIVES INT. MED., 1913, xii, 621. See later, Experiment 4.

By decreasing the total vaccinating dose we were finally able to estimate with certainty that a given amount of one type of vaccine would protect for five or six weeks; with this point determined, it was easy to compare the immunizing value of other preparations with this one. In nearly all experiments, two or more control normal animals have been injected, although their use is not absolutely imperative since the less well protected animals become carriers and the percentage of carriers in each series gives the basis of comparison.

DETERMINATION OF THE TYPHOID VACCINE OF ELECTION FOR IMMUNIZATION

The carrier condition was at first determined in the immunized and control animals by cultures taken from the blood ten and twenty days after inoculation; the results were checked by the post-mortem condition and cultures from the bile when the animals died, or in the case of survivors by chloroforming them from fifty to seventy days later. A later method which gives quicker results is to chloroform all the survivors at the end of ten or fourteen days and make cultures from the blood and bile. A perfectly immunized animal is one that gives negative blood-cultures and at death presents no lesions particularly of the gall-bladder and gives negative cultures from the bile.

Throughout our experiments it has been our effort to kill our cultures of *B. typhosus* in a manner designed to obviate, so far as possible, the deteriorating effect of heat that has been evidenced by the work of numerous observers mentioned before. The introduction of heat, at all events for the purpose of obtaining sterile cultures, seems to us quite unnecessary, and, as one of us pointed out a number of years ago,⁵⁹ cultures of the dysentery bacillus may be sterilized and preserved by the simple addition of 0.5 per cent. trikresol or phenol, without destroying their toxic and antigenic properties to the same degree as in corresponding cultures that are first killed by heat at 60 C. (140 F.) and then preserved by trikresol. Very little attention appears to have been paid to this observation, although we believe that several have utilized the method with advantage, killing the cultures either with liquor formaldehydi or with trikresol. In the present series of experiments we have desired not only to kill the bacteria with little harm but also to collect and dry them in order that the dosage employed might be reckoned from weighed amounts of the dried bacilli, which had been previously ground in an agate mortar. For this purpose, as already stated in Experiment 1, we

59. Gay: Vaccination and Serum Therapy Against the Bacillus of Dysentery, Univ. Penn. Med. Bull., 1902, xv, 307.

precipitate our various bacterial suspensions with equal parts of absolute alcohol, which accelerates the centrifugalization and the drying of the preparation. If the culture is to be sensitized, a definite amount of a potent, immune serum from the rabbit is previously added, the dosage being estimated in accordance with the agglutination titer of the serum in question and the number of bottles from which it was obtained, the amount being 1 c.c. of a serum that agglutinates the organism in question in a dilution of 1:20,000 to the suspended growth from each of the seven Blake bottles. After standing for two or three hours in an incubator at 37 C. (98.6 F.), the sensitized culture is left in the ice-chest over night, centrifugalized, the supernatant fluid removed, the sediment washed in the original amount of normal saline, recentrifugalized, and the volume restored to normal with sterile saline. This washed sensitized culture is then flocculated by adding an equal volume of absolute alcohol, dried, ground and weighed as in the unsensitized culture.

TABLE 2.—SUMMARY OF EXPERIMENT 4

Rabbit No.	Vaccination	Interval Before Inoculation Weeks	Agglutinins	Result
9	Plain Vaccine 1	12	1:100	Recovered
10	Plain Vaccine 1	12	Neg. 1:100	Carrier
12	Plain Vaccine 1	12	1:500	Carrier
13	Sensitized No. 1	12	Neg. 1:100	Recovered
14	Sensitized No. 1	12	Neg. 1:100	Recovered
Control 71	Carrier
Control 72	Carrier

We first compared the protective value of sensitized and unsensitized cultures. The results of our first experiment (Experiment 4) may be given, although few animals are involved, because the results were particularly clear-cut. Similar results will be shown in a later experiment in connection with other preparations.

EXPERIMENT 4.—Dried sensitized and unsensitized vaccines were prepared from the same growth of *B. typhosus* 5 (Army and Navy, Dorset strain) in the manner described above. Four rabbits were given 0.56 mg. of the dried unsensitized vaccine intravenously in 1 c.c. carbolated saline solution for three successive days. Two other rabbits were given the same amounts of the same vaccine *sensitized* in the same manner. One of the first series (plain vaccine) died by accidental injury. Three months after the last injection, the agglutination titer of the immunized rabbits was tested and they and two control animals of similar weights were given the standard test dose of living blood-agar typhoid bacilli ($\frac{1}{2}$ culture *B. typhosus* 3c) intravenously. The results are summarized in Table 2.

This experiment indicates the superior value of a sensitized vaccine over an unsensitized vaccine in producing a durable immunity. It further shows the unreliability of agglutinins as indicating resistance to actual infection with the typhoid bacillus. As we have already noted in the introduction, other observers have found that sensitized bacteria produce less agglutinins than untreated bacteria, but more tropic or sensitizing antibodies.

The method which we have described for preparing and grinding the vaccine is essentially similar to the one described by Besredka for the preparation of endotoxins, and it may, indeed, be shown in the case of the untreated vaccine that the supernatant fluid contains the greater part of the toxic substances of the whole vaccine (Besredka,⁶⁰ Steinitzer⁶¹). It seemed to us important to determine at this point whether or not the supernatant fluid of such a ground and suspended preparation of typhoid bacilli, or the sediment of bacterial bodies left on centrifugalizing this mixture contains the immunizing principle. In the following experiment (Experiment 5), in which the immunizing value of the two preparations is shown, it is very evident that it is the sediment or bacterial bodies deprived to a great extent of toxicity of the whole vaccine which is the essential immunizing principle. This immediately suggests the advisability, from the point of view of symptoms produced and of protecting value assured, of utilizing simply the remnants of the bacterial bodies obtained in such a vaccine rather than the whole vaccine itself. Experiments designed to test out the comparative value of the whole vaccine, on the one hand, and the sediment on the other, show very distinctly that the sediment possesses not merely as much but apparently actually more immunizing value than the whole vaccine (Experiment 6).

EXPERIMENT 5.—In this experiment are combined the results obtained from two different plain unsensitized vaccines, Vaccine 1, which has already been described (Experiment 4), and Vaccine 8 prepared in the same way but from blood-agar cultures grown on a blood-agar medium. The weighed, dried vaccine was in each case suspended in the proper amount of 0.5 per cent. carbolated saline solution and allowed to stand for forty-eight hours with thorough shaking at intervals. The suspended vaccine was then centrifugalized thoroughly, leaving an opalescent *supernatant* fluid (endotoxin) and a grayish white *sediment* of ground bacterial bodies. The supernatant fluid was removed and the sediment restored to the original volume by adding fresh carbolated saline solution. Rabbits were then vaccinated in series with the supernatant and sediment, respectively. The dosage of vaccine preparations given in three doses on successive days (volume 1 c.c. on each injection) and the resultant

60. Besredka: Etudes sur le bacille typhique et le bacille de la peste, Ann. de l'Inst. Pasteur, 1905, xix, 477.

61. Steinitzer: Ueber die Toxine (Endotoxine) der Typhusbazillen; Kraus and Levaditi, Handbuch der Immunitätsforschung, 1908, i, 193.

condition obtained on testing the animals with the standard dose of living culture is summarized in Table 3.

Table 3 shows the marked superiority of sediment over supernatant fluid in producing immunity from infection; all of the six sediment-treated animals recovered, whereas only one of eight supernatant animals failed to become carriers. The further unreliability of the agglutinin test is shown by the fact that in the first series the supernatant gives higher agglutinins but no protection.

TABLE 3.—COMPARISON OF IMMUNIZING POWER OF THE SUPERNATANT AND SEDIMENT DERIVED FROM PLAIN TYPHOID VACCINES*

Rabbit No.	Vaccination Employed	Interval Before Testing Months	Agglutinins	Result
21	Supernatant of Vaccine 1. Total dosage, 1.68 mg. in three days	2	1: 50	Carrier
22	2	1: 400	Carrier
26	2	1: 200	Carrier
27	2	1: 500	Carrier
23	Sediment of Vaccine 1; 1.68 mg. in three days	2	1: 100	Recovered
28	2	1: 400	Recovered
29	2	1: 100	Recovered
85	Supernatant of Vaccine 8; 10 mg. in three days	Weeks 6	Carrier
86	6	Recovered
87	6	Carrier
88	6	Carrier
73	Sediment of Vaccine 8; 10 mg. in three days	6	Recovered
75	6	Recovered
76	6	Recovered

*The control rabbits are purposely omitted for simplification; they were positive, as is the rule.

One of our most recent experiments summarizes the facts that have been adduced as to the most protective preparation, and further brings out the fact, already stated, that the sediment actually protects better than the whole vaccine. In comparing the whole suspended vaccine with the sediment derived from such a vaccine, it should be noted that every technical error necessary to produce the sediment

from the whole vaccine would tend to lessen the amount of material in the sediment, and the superior immunizing value of the latter is therefore so much the more striking. The results of this experiment are summarized in Table 4. The preparations used were prepared as already described, the preparations all being derived from an original suspension of *B. typhosus* 4 (recently isolated stain, October, 1913), grown on agar (Vaccine 20).

TABLE 4.—COMPARISON OF WHOLE SUSPENDED VACCINE WITH SEDIMENT *

Rabbit No.	Vaccination Employed	Interval Before Testing Days	Result
262	0.5 mg. whole unsensitized Vaccine 20 in three days intravenously	32	Carrier
265	32	Recovered
266	32	Carrier
263	32	Died before testing
264	32	Died before testing
267	0.5 mg. sediment unsensitized Vaccine 20 in three days intravenously	32	Carrier
268	32	Recovered
269	32	Carrier
270	32	Recovered
271	32	Carrier
273	0.5 mg. whole sensitized Vaccine 20 in three days intravenously	32	Died acutely (anaphylaxis)
274	32	Killed by injury
275	32	Recovered
276	32	Recovered
277	0.5 mg. sediment sensitized Vaccine 20 in three days intravenously	32	Carrier (bile culture only positive)
278	32	Recovered
279	32	Recovered
280	32	Killed by accidental injury
281	32	Recovered

* Controls again purposely omitted.

The points which we have already brought out are emphasized in this experiment. Thus the percentages of recovery are 33 for whole unsensitized, 60 for whole unsensitized sediment, 66 for whole sensitized, and 75 for sensitized sediment. The first series also shows the greater toxicity of the whole culture as compared with its sediment.

We seem so far to have determined, then, that killed, sensitized cultures are somewhat more protective than plain, unsensitized, killed cultures of the typhoid bacillus and that the protection is due to the bacillary sediment and not to the supernatant endotoxic fluid. These sediments, moreover, seemed distinctly more protective than the whole vaccines from which they were derived.

In view of the assertions of Metchnikoff and Besredka, it remains to determine by our method whether or not living, sensitized cultures have any superior immunizing value over sensitized cultures killed

TABLE 5.—COMPARISON OF LIVING SENSITIZED WITH DRIED WHOLE SENSITIZED VACCINES

Rabbit No.	Vaccination Treatment	Interval Weeks	Result
171	1 mg. living sensitized culture* intravenously in three doses, on three successive days.	7	Recovered
172	7	Carrier
173	7	Carrier
174	7	Carrier
175	10	Recovered
176	10	Recovered
179	1 mg. dead sensitized culture intravenously in three doses, on three successive days.	7	Carrier
180	7	Recovered
181	7	Carrier
182	7	Carrier
184	10	Carrier
185	10	Recovered
186	10	Recovered

*Computed from same culture dried.

by alcohol. For this purpose we have compared the living sensitized culture first with whole dried sensitized culture and then the living sensitized culture with the sensitized sediment. In these experiments the original bacterial suspension is sensitized, washed, and divided into two parts, one of which is left intact and the other precipitated by alcohol, dried, ground, weighed, and suspended in the original amount of fluid. Here again technical errors would militate against the immunizing strength of the dried culture, particularly when the latter is carried further to the sediment preparation. The results of two such experiments are summarized in Tables 5 and 6.

The first experiment shows that there is little or no difference to be shown between the immunizing powers of living sensitized cultures

as compared with sensitized cultures killed by alcohol. The second experiment shows the immunization by dead sensitized sediment to be distinctly superior to that by living sensitized cultures.

PROTECTIVE VACCINATION IN HUMAN BEINGS BY MEANS OF THE
MODIFIED SENSITIZED CULTURE

Our experimental results had reached a stage nearly a year ago when we felt justified in recommending the whole sensitized culture that we have described for use in protecting human beings against

TABLE 6.—COMPARISON OF LIVING SENSITIZED CULTURE WITH DEAD SENSITIZED SEDIMENT *

Rabbit No.	Vaccination Treatment	Interval Weeks	Result
296	1 mg. living sensitized culture intravenously in three doses on three successive days.	†	
289	7	Carrier
290	7	Carrier
291	7	Recovered
292	7	Carrier
295	7	Carrier
293	8	Carrier
297	10	Recovered
305	1 mg. dead sensitized sediment intravenously in three doses on three successive days.	†	
299	7	Recovered
300	7	Recovered
302	7	Carrier
303	7	Recovered
304	7	Recovered
298	8	Carrier
301	9	Carrier
306	9	Recovered

* Of group A, two animals, or 25 per cent., recovered, and six animals, or 75 per cent., remained carriers. Of group B, five animals, or 62.5 per cent., recovered, and 3 animals, or 37.5 per cent., remained carriers.

† Died before testing.

typhoid fever. It seemed demonstrated from the work on rabbits that the sensitized vaccine, washed, precipitated, killed with alcohol, and ground would protect at least as well and probably better than the ordinary vaccine that had not been sensitized, and we anticipated much fewer untoward symptoms following vaccination by the use of this sensitized vaccine. This absence of untoward effects has been fully justified in experience. Up to the present time over 1,000

students in the University of California have been immunized by Professor Force of the Department of Hygiene. The absence of reaction or the mildness of reaction has been very striking. Force⁶⁷ has already reported on the first 261 cases treated by this method, and less complete analysis of the subsequent cases agrees with the results obtained in the smaller number. In these 261 cases—apart from the local symptoms of induration, redness, and slight tenderness for one or two days—general symptoms, even of a mild grade, were very seldom observed. The vaccine was administered on alternate days, the treatment being completed within a week. The very fact that it was possible to give the second and third vaccinations on the second and fourth days after the first indicates strongly that even the local condition was so mild as to allow the subsequent inoculations. The dosage at first employed was $\frac{1}{16}$ mg. of dried culture suspended in 0.5 per cent. carbolated salt solution, corresponding, as we have determined, to 500 million bacteria. Since this produced little or no reaction, we have since increased it by one-half ($\frac{3}{32}$ mg.). In only six cases in the total number of inoculations practiced (671) did the temperature rise above 38 C. (about 100 F.). The symptoms were somewhat more pronounced in women than in men. In two cases of arrested tuberculosis the reaction was more marked and in a few cases that gave a history of previous typhoid fever the reaction was also slightly more pronounced. More recently we have employed the sensitized sediment vaccine in accordance with the experimental results that have just been obtained, which show conclusively that this substance is more immunizing than the whole sensitized preparation. This sensitized sediment gives even fewer reactions than the whole sensitized vaccine. Thus in a recent series that Force has vaccinated with sensitized sediment, using a dosage corresponding to 750 million bacteria, including 672 separate injections, malaise was noted in 1.8 per cent.; fever (above 99 F.) in 4.5 per cent.; pain in back in 2.4 per cent.; pain in head in 9 per cent., and pain in the arm in 3 per cent. In cards returned to us from 231 outside patients to whom the vaccine was distributed, 215 report total absence of local and general reactions (93 per cent.). Ten cases were marked as having shown generalized symptoms and three cases of typhoid recoveries and three patients who had been previously vaccinated also showed slight symptoms. These reactions may be compared with the following: Hartsock⁶² obtained a mild reaction, by which he specifies "temperature up to 100 F. with slight general reaction, malaise, and considerable local tenderness," in 83 per cent. of men treated with U. S.

62. Hartsock, F. M.: Antityphoid Vaccination, Jour. Am. Med. Assn., 1910, liv, 2123.

Army vaccine. Hatchel and Stoner⁶³ in 1,326 cases vaccinated with a polyvalent vaccine found malaise in over 58 per cent. and fever in 43 per cent. Albert and Mendenhall⁶⁴ used the U. S. Army strain in regular doses and intervals and found that the local reaction lasted for from three to five days. Fever as high as 103 F. was usual and malaise, headache, and insomnia were common.

Auché and Chevalier⁶⁵ have reported a notable failure of prophylactic typhoid vaccination in a person who received three injections in January, and in July came down with a well-defined case of typhoid fever. We agree with the authors that such cases should be reported as indicating the limitations of the method. Fornet has collected further cases in which the protection was not produced. In our early series of cases among the students treated with whole monovalent sensitized vaccine, in which it has been possible to follow the results with great accuracy, there has been one case of typhoid fever among the vaccinated persons five months after treatment; the recovery was uneventful. These instances go to prove that further perfecting of any method in vogue is necessary. Both Wassermann²⁴ and Vincent¹⁵ have suggested the use of polyvalent vaccines; although the typhoid bacillus has been regarded as an unusually fixed species, the occurrence of minor biologic peculiarities, particularly in the fermentation of sugars, has been noted in the collection of organisms which we have studied. In accordance with these facts, as well as with the results that we have detailed, we have recommended a polyvalent sensitized sediment for prophylactic immunization against typhoid fever in human beings. The organisms, five in number, which have been used to produce the polyvalent mixture was isolated from recent cases of typhoid fever in the vicinity. An additional advantage in the use of sensitized over unsensitized cultures is that a strain of the typhoid bacillus may be used as in our polyvalent vaccine without producing any more symptoms than with a monovalent vaccine. It is particularly unnecessary to seek a "mild strain" as in the case of the U. S. Army vaccine. Our various strains are grown separately on Blake flasks, and the suspended cultures mixed and treated with a polyvalent immune serum obtained by immunizing rabbits with each of the strains in turn. This polyvalent sensitized sediment vaccine is now being manufactured and distributed free of cost by the California State Board of Health to any physicians in the state who may apply.

63. Hatchel, F. W., and Stoner, H. W.: Inoculation Against Typhoid, *Jour. Am. Med. Assn.*, 1912, lix, 1364.

64. Albert and Mendenhall: Reactions Induced by Antityphoid Vaccination, *Am. Jour. Med. Sc.*, 1912, cxliii, 232.

65. Auché and Chevalier: Un cas d'insuccès de la vaccination antityphique, *Jour. de méd. de Bordeaux*. 1913, xxiii, 371.

THE TYPHOIDIN REACTION

One of the greatest difficulties that has been present in determining the protective value of typhoid immunization as a whole has been the impossibility of determining the protection of a given group of persons by other means than the careful study of morbidity statistics among vaccinated people over a long period of years (Firth⁶⁶). This difficulty delayed the final acceptance of typhoid immunization for at least eight years (1896-1904). Still less have we any means of determining whether or not a given person who has been vaccinated is actually protected against typhoid fever. We have already described in full the skin-test which Gay and Force⁶⁷ have employed as of presumptive value in testing resistance to typhoid infection.

A new summary of our results with the typhoidin may be of interest: In forty-four cases giving a definite history of typhoid fever from four and one-half months to forty-one years previously, forty have given a clear-cut skin-test to the concentrated growth of the bacillus. Of the four negative tests, two gave a strong skin-reaction to a similar preparation made from *B. paratyphosus A.* which we have found not to occur in anything like the same intensity in those cases that react to typhoidin. Only one of the remaining negative cases was further tested with the paratyphoidin solution and therefore only one of two cases was unexplainably negative and 95 per cent. (forty of forty-two) positive. We have a growing set of observations on comparative tests with two paratyphoidin solutions (A and B) in recovered typhoid and paratyphoid cases, and in those vaccinated against typhoid, which we may wish to present at a later time; they apparently show certain interesting group reactions.

Of the controls giving no history of typhoid fever, thirty-eight out of forty-four, or 86 per cent., have been clearly negative. Only five of these control cases (11 per cent.) reacted distinctly positively, and we believe may be explained as cases of aborted or mild, undiagnosed typhoid fever. This explanation is rendered probable by the observation, brought to our attention by Dr. Edward von Adelung, of a perfectly controlled clinical experiment on this subject. The observer, Dr. von Adelung, was a member of a family party of four which visited Germany nineteen years ago. About two weeks after drinking at a suspected water source, two of the members came down with a fever which ran the typical course of typhoid and was so diagnosed. The other two members of the party at the same time

66. Firth: A Statistical Study of Antienteric Inoculation, Jour. Royal Army Med. Corps, 1911, xvi, 589.

67. Gay, F. P., and Force, J. N.: A Skin Reaction Indicative of Immunity Against Typhoid Fever, Studies in Typhoid Immunization III, THE ARCHIVES INT. MED., 1914, xiii, 471.

had mild symptoms, lasting one and three days, respectively, and consisting of headache, fever, malaise and a sensation of flushing, which they regarded as abortive typhoid. Neither of the latter two persons gave any other history of typhoid fever. When the skin-reaction was applied to the two cases that had run the typical typhoid course and to the two that had passed through the "abortive" attacks, all gave positive results. This well-controlled, natural experiment would seem to prove that the supposedly normal cases that react to typhoidin may well be those that have had abortive or undiagnosed typhoid fever in the past.

Our experience with the skin-reaction in those who have been vaccinated with various typhoid vaccines, including our own, has shown us that the majority of those who have been treated within a year and a half or two years may be expected to give a positive reaction, provided always that the last injection has been given at least a month previously. After two years the reaction is less likely to be positive. This experience with the skin-test would correspond very closely with what has been found clinically to be the usual duration of artificial typhoid immunity. We have felt justified, then, in recommending to students who have taken the typhoid vaccine previously that they should have a skin-test applied subsequently at intervals and that if it turned out to be negative they should repeat the treatment.

Since our last communication on the typhoidin test, we have been led to modify the preparations of the solutions employed. We found that some of the original preparation of typhoidin after four or five months failed to produce a positive reaction in cases in which we had been led to expect it. That this failure was due to a deterioration in the product was evidenced by subsequent positive results obtained in six such cases by means of a new preparation. Although such deterioration does not occur for a considerable time with tuberculin, it is known to take place in mallein and abortin. In the latter two cases and in tuberculin as well, better reactions have been obtained with preparations purified by alcohol precipitation and dried (compare Haring⁶⁸ and Meyer and Hardenbergh⁶⁹). A similar preparation has been made by precipitating the original typhoidin with twenty volumes of alcohol, filtering, washing with absolute alcohol and ether, and then drying on porcelain plates over a vacuum with sulphuric acid. The control solution, 5 per cent. glycerin-bouillon evaporated to one-tenth volume, was treated in a similar manner. Ten c.c. of the

68. Haring, C. M., and Bell, R. M.: *The Intradermal Test for Tuberculosis in Cattle and Hogs*, Univ. Cal. Pub. Agric., 1914, Bull. 243, p. 94.

69. Meyer and Hardenbergh: *On the Value of the "Abortin" as a Diagnostic Agent for Infectious Abortion in Cattle*, Jour. Infect. Dis., 1913, xiii, 351.

original typhoidin yielded 0.78 gm. of dried powder when the culture had been grown for five days before evaporating. The dried powder from the same amount of a control solution gave only 0.5 gm. There is every reason to believe that this dried typhoidin will keep its potency undiminished for at least a considerable time. Our observations, however, extend only to its trial over a period of two months. We dissolve a small amount in carbolated saline equivalent to the original volume of concentrated typhoidin or even to a double concentrated solution and find that when kept in a cool place, it gives very good reaction in typhoid immunes for at least a month.

Our suggestion and growing belief that this skin reaction with typhoidin is a real measure of the protection that the person enjoys against typhoid fever is strengthened by observations on our immunized rabbits. We have already shown that the agglutinin titer is no indication of the resistance of a given animal to infection, and observations on the Widal reaction in man tend to the same belief (Ruediger and Hulbert⁷¹). The typhoidin reaction, on the other hand, is positive in that category of persons who are known to be protected against typhoid fever, namely, typhoid recoveries; it does not occur in persons who give no history of the disease, except in a small percentage that may reasonably be suspected of having had an abortive attack. The reaction further occurs in the majority of those persons who have been vaccinated against typhoid within the last two years and then gradually disappears. We had scarcely hoped to show differences in typhoidin reaction between incompletely and perfectly immunized rabbits as tested by our method of infection, which is, after all, a violent one, but our results in this respect have exceeded our anticipations. It is, however, easy to demonstrate fundamental differences between normal and immunized rabbits by the intradermal reactions. Cutaneous reactions were first tried, but abandoned as unsatisfactory.

With the intradermal reaction, performed by producing a tiny bleb under the skin by a short needle carrying typhoidin, a distinctive reaction is produced in rabbits that have been artificially immunized, but not in controls. In each case a patch on the abdominal surface is shaved the day before the test and two blebs produced under the epidermis by injecting in the one concentrated glycerin bouillon and in the other typhoidin. In the normal animal there is little or no difference between control and typhoidin spot. Both are usually surrounded by a red zone of a few millimeters, and there may be slight induration. In the animal that has previously been treated by typhoid

71. Ruediger and Hulbert: Is Dried Blood as Reliable as Fresh Serum in Making the Widal Test? *Am. Jour. Pub. Health*, 1914, iv, 113.

vaccines there is a sharp difference between the two spots. The typhoidin spot, as early as five hours, but with increasing regularity and intensity to twenty-four hours, becomes surrounded by a red areola, is indurated, and quickly forms a firm, hard nodule with a yellowish, slightly softened center, from 2 to 5 mm. in diameter. This nodule persists at least for several days and may last two or three weeks. It is characterized histologically by an infiltration of lymphoid cells with which are mixed a few polymorphonuclear leukocytes.

Five normal rabbits tested in this manner gave negative reactions. Of thirty-five more or less perfectly immunized rabbits, twenty-six (74 per cent.) reacted positively. The differences in reaction between animals effectively immunized against our method of producing carriers and those which are not so fully protected is a relative matter when viewed in the aggregate, but in individual experiments is more striking. Thus, dividing our treated animals in respect to a negative or positive intradermal test and then comparing the results with their carrier condition or recovery on infecting the following day, we find that of nine with a negative intradermal test, seven, or 78 per cent., became carriers; of twenty-four with a positive intradermal test, thirteen, or 54 per cent., became carriers, a distinct, though only relative indication of agreement between the test and the absolute protection.

In Table 4 is given the comparative immunizing value of four different vaccine preparations. Before the test inoculation, the intradermal test was applied to these animals with the following results as compared with the eventual carrier conditions:

Group 1, vaccinated with whole unsensitized vaccine (three animals): intradermal test, 3 negative; carriers produced, 2; recovered, 1.

Group 2, vaccinated with unsensitized sediment (five animals): intradermal test, 4 positive; carriers produced, 3; recovered, 2.

Group 3, vaccinated with whole sensitized vaccine (three animals): intradermal test, 3 positive; acute death (anaphylaxis), 1; recovered, 2.

Group 4, vaccinated with sensitized sediment (four animals): intradermal test, 4 positive; carriers produced, 1; recovered, 3.

These results show a distinct relation between a positive intradermal test and recovery and between a negative intradermal test and establishment of the carrier state.

MECHANISM OF THE TYPHOIDIN REACTION

We do not intend, at this time, to discuss fully the experimental evidence and the hypotheses that have been brought forth to explain the generalized and localized reactions that follow the injection or application of bacterial extractives like typhoidin in infected or immunized individuals. Of these reactions, the one to tuberculin has natur-

ally been most studied. Some of the most interesting and debatable questions that seem to have arisen in connection with the tuberculin reaction are, first, whether or not it is in reality a reaction exactly similar to the anaphylactic reaction with serum, and second, whether it is due to an interaction of antigen and antibody accompanied, it may be, by alexin fixation (Wassermann and Bruck). As regards the local, cutaneous test we have the further possibility that the antibodies, hypothetically furnished to unite with the antigen, may be furnished from the general circulation or due to local cellular response (von Pirquet). There is certainly evidence in the case of typhoid recoveries that the protection is cellular rather than humoral, and the relative inefficiency and short duration of artificial vaccination against typhoid fever may well be due to the fact that the immunity is not sufficiently cellular in type.

It seemed possible to attack certain of these problems in relation to the localized typhoidin test. The first question that arose was whether or not the reaction was due to the local combination of antigen and antibody. As has already been shown, the sensitized vaccine which we have employed gives less reaction, both general and local, than the untreated vaccine when used in the prophylactic treatment of human beings. We should not, therefore, expect that the local application of a sensitized vaccine in normal individuals would call forth any, or at any rate, not so marked a response as the untreated vaccine. And yet, it was argued, if the local typhoidin reaction in an immunized individual is really due to a combination of antigen and antibody, sensitized vaccine, which is just such a combination, might produce a local reaction in a normal individual.

EXPERIMENT 6.—Suspensions of unsensitized, dried Typhoid Vaccine 4 and of sensitized, dried Typhoid Vaccine B were made in the proportions of 5 mg. of vaccine to 1 c.c. of carbolated saline solution.

On normal rabbit 319 were produced intradermally small blebs of the foregoing sensitized and unsensitized suspensions. Twenty hours later the following condition was observed:

"Vaccine 20 bleb (unsensitized) measures 3 by 5 mm., is not markedly raised, and has no yellowish center.

"Vaccine B bleb (sensitized) measures 6 by 5 mm., is surrounded by an areola that is indurated, markedly higher than the other point, and with large, firm, raised yellowish papule, which resembles in all respects the positive skin-reaction obtained in immunized animals except that the surrounding areola is not quite so extensive."

Normal Rabbit 322, treated in the same manner, showed slightly more reaction about the unsensitized spot than the sensitized, but no distinct papule was present as in Rabbit 319.

A skin test with the two preparations was then tried on two normal, unvaccinated human beings. No reaction was noted with either spot. Since the intradermal test is recognized as being more sensitive, it was next tried on normal persons.

Thus, when comparative intradermal tests with sensitized and unsensitized vaccine were made on two normal rabbits, one of the two showed a much more marked reaction to the sensitized vaccine than to the unsensitized, the sensitized vaccine reaction resembling in all respects that produced in an immunized animal by the injection of typhoidin.

Of four human beings that were similarly tested by intradermal blebs, three showed a very characteristically increased reaction to the sensitized vaccine over the unsensitized vaccine. In only one case were the reactions produced by the two suspensions similar, and both were so marked as to suggest the possibility of an overlooked typhoid history. We regard this experiment, then, as distinctly indicating that the local reaction to typhoidin is due to an interaction of antigen and antibody.

TABLE 7.—NORMAL PERSONS TESTED BY INTRADERMAL TEST WITH SENSITIZED AND UNSENSITIZED TYPHOID VACCINES*

No.	Reaction to Sensitized Vaccine (5 mg. to 1 c.c.)	Reaction to Unsensitized Vaccine (5 mg. to 1 c.c.)
1	24°. Large indurated area 80 by 100 mm.	Same as other bleb
2	18°. Red area 50 by 58 mm. (Foregoing solutions diluted one-tenth concentration) (0.5 mg. to 1 c.c.)	2.2 by 2.2 mm. Less red
3	24°. Demarcated, red, slightly indurated area, painful 7.1 by 5.4 mm.	Indefinite, slightly red area, 4.2 by 2.1
4	24°. Red, demarcated area 6.2 by 5.2 mm.	Less red, not demarcated, 4.9 mm.

*The tests were applied by making blebs, one on each upper arm.

One further point on the mechanism of the typhoidin skin-test has been brought out by experiments on rabbits. Having reached a logical assumption that the skin-test is due to the interaction of antigen and antibody in the immune individual, it remained to show whether the antibody furnished for this combination comes from the circulating blood or is due to local tissue reaction. If the first hypothesis is true, immune serum should transfer the reactionability to a normal animal and an immune animal that reacts should at least partially lose this power if exsanguinated and transfused with normal rabbit blood. Both these conditions were found possible of realization.

EXPERIMENT 7.—The results of this experiment may best be described in parallel columns (Table 7).

This experiment, as well as another one carried out under the same conditions and with the same results, shows clearly that the abstraction of blood from an immune animal that reacts strongly to an intradermal test of typhoidin renders it almost completely insusceptible, whereas a control immune reacts a second time on corresponding days. The blood of the immune animal transfers the susceptibility to the typhoidin reaction to a normal animal, which has previously reacted negatively. We may conclude, therefore, that in the case of immunized rabbits, and perhaps also in the case of human beings artificially immunized against typhoid fever, the skin-reaction may be accounted for by the interaction of the antigen with antibodies in the circulating blood. We do not wish at all to suggest that the skin-reaction in typhoid recoveries is due to the same mechanism. The absence of

TABLE 8.—TRANSFER OF REACTIBILITY TO SKIN REACTION BY TYPHOIDIN

Date	Typhoid Immune Rabbit 298	Typhoid Immune Rabbit 293	Normal Rabbit 329
April 7...	Intradermal test applied	Intradermal test applied	Induration test applied
April 8...	Reaction strongly positive	Reaction strongly positive	Reaction negative
April 9...	Bled for 50 c.c. and rapidly transfused from a normal rabbit	
April 10...	Intradermal test applied	Intradermal test applied	Given 20 c.c. serum from Rabbit 293
April 11...	Reaction strongly positive	Reaction negative; slight induration; no papule	Intradermal test applied
April 12...	Reaction distinctly positive and increasingly so to seventy-two hours; induration; papule and yellow center.

demonstrable antibodies in the majority of these cases, as well as their superior type of immunity, would lead one to think that the skin reaction may be cellular rather than humoral in character.

SUMMARY AND CONCLUSIONS

The history of the development of artificial immunization against the typhoid bacillus in animals and human beings is reviewed, and some of the many preparations of the typhoid bacillus used for this purpose are discussed. Particular attention is drawn to the reputed advantages of living sensitized typhoid vaccine (Besredka) as opposed to other types of vaccine. The many vaccines that are still being

advocated indicate that the best vaccine has not yet been found and that the best method of proving which is the best vaccine has not been determined.

In the present article we explain again the method of testing typhoid immunity by means of an artificial typhoid-carrier state in the rabbit, and again advocate it as the best test for this purpose short of experimentation on anthropoid apes. We find this method admirably suited to indicate slight differences in typhoid preparations as regards their ability to produce an immunity against a subsequent typhoid carrier condition produced by intravenous injection of living typhoid bacilli. In successive series of experiments we have been able to show by this method, first, that untreated bacteria, killed and precipitated by alcohol, dried, ground and weighed, do not protect so well as typhoid bacilli that have been sensitized by an immune serum and subsequently treated in the same manner. Second, it has been possible to show in the case of the unsensitized, dried bacteria that the sediment of bacterial bodies freed from the supernatant, endotoxic fluid as prepared from these dried cultures contains the immunizing principle almost in its entirety. The sediment of either sensitized or unsensitized cultures protects not only better than the supernatant fluid from these sediments, but actually better than the whole, unseparated mixture. Third, we find that alcohol-killed, sensitized cultures protect almost as well as living, sensitized cultures and that the sediment of alcohol-killed, sensitized cultures protects better than living, sensitized cultures.

Sensitized cultures of the typhoid bacillus, whether whole or as sediment, produce little or no reaction in human beings with the possible exception of those who have previously suffered from typhoid fever or have been immunized. By comparing our results with those of certain other observers, we conclude that a considerable degree of reaction, both local and general, is avoided by the use of these sensitized cultures, which possess the further advantage, so far as our experimental work can determine, of producing a more durable type of immunity.

We recommend vaccination at short intervals (two days) in human beings, which is rendered quite possible with the mild vaccine we employ, and is evidenced from animal experimentation, as giving rise to less toxic effect and to fully as durable an immunity as vaccination at longer intervals. This type of vaccination has the further advantage of completing the prophylactic treatment of three injections within a week.

We further believe that a polyvalent vaccine derived from strains of the typhoid bacillus isolated in the vicinity of cases that are to be

treated is advantageous, judging largely from the work of other observers. A further advantage in the use of sensitized cultures is that a polyvalent vaccine, no matter how recently the strains may have been isolated, is also almost entirely free from untoward effect. We have no direct experimental evidence bearing on the superior immunizing value of such a polyvalent vaccine. The dosage of vaccines in human beings which we recommend is empirical and based on the usage of other experimenters.

We recommend, then, for prophylactic immunization against typhoid fever, three injections of the sediment of a dried, ground, sensitized culture of several local strains of the typhoid bacillus mixed together, given at two-day intervals and in a dosage of $\frac{3}{32}$ mg. of the original dried culture, which corresponds, as has been determined, to a dosage of approximately 750 million living typhoid bacilli.

Our own experimental and clinical results, as well as the work of many other investigators, leads us to regard the agglutination reaction with the serum of immunized animals and human beings as by no means indicative of the degree of protection afforded against infection with the typhoid bacillus. Of far more prognostic significance is the skin test with the typhoidin solution, which Gay and Force have recently described. Further summaries of the results with this test show that this reaction is positive in the majority of recovered cases of typhoid (95 per cent.), even as far back as forty-one years, and that it occurs in only about 11 per cent. of persons who give no history of typhoid or of typhoid immunization. Distinct evidence is brought forth that indicates that these supposed normals reacting to typhoidin have suffered from an abortive or undiagnosed typhoid fever. Persons immunized with various types of typhoid vaccine react in the majority of cases for about two years and then become more frequently negative. We regard a negative skin-test after vaccination as indication for revaccination.

That a positive typhoidin test bears a distinct relation to protection against typhoid fever is further indicated by similar reactions produced intradermally in our immunized rabbits. In spite of our more violent method of artificial infection as against natural infection in typhoid fever, we find that animals that resist becoming carriers show a higher percentage of positive intradermal tests to typhoidin administered the day before; whereas those that are not immunized sufficiently to resist the carrier state more frequently show a negative typhoidin reaction.

We suggest the use of dried, alcohol precipitate from typhoidin solution as a stock from which the test solution may be prepared, since it has been found that the original typhoidin solution deteriorates within a few months.

Experiments dealing with the mechanism of the local typhoidin reaction indicate that it is due to an interaction of antigen and antibody, as is shown by the fact that the *intradermal* test of a sensitized vaccine will produce a characteristic reaction in human beings, whereas the corresponding amount of an unsensitized vaccine produced no such reaction. The paradox of producing a more severe local reaction with the sensitized vaccine, which has been found to produce less violent reactions when used for immunization, is only apparent. Further experiments with rabbits indicate that in the condition of artificial immunization against the typhoid bacillus, the antibodies which unite with the antigen to produce the local test are in the circulating blood, as is indicated by the passive transfer to a normal rabbit by means of serum from an immune rabbit of susceptibility to this reaction. This is further evidenced by the extraction of blood from the immunized rabbit and a corresponding loss of reaction. We do not regard this experiment, however, as indicating the circulatory nature of the antibodies in the recovered typhoid persons who almost invariably react to typhoidin.

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