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I. DETERIORATION OF TYPHOID VACCINE

By G. W. McCOY and IDA A. BENGTSON

II. STANDARDIZATION OF GAS GANGRENE ANTITOXIN

By IDA A. BENGTSON

III. POTENCY OF BACTERIAL VACCINES SUSPENDED IN OIL (LIPOVACCINES)

By IDA A. BENGTSON

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CONTENTS.

ILLUSTRATIONS.

I. DETERIORATION OF TYPHOID VACCINE.[a]

By G. W. McCoy, *Director*, and Ida A. Bengtson, *Bacteriologist*, Hygienic Laboratory, U. S. Public Health Service, Washington, D. C.

An experiment was carried out over a period of two and one-half years to determine the effect of various storage temperatures on the agglutinin-producing properties of typhoid vaccine.

The vaccine with which the tests were carried out was one made according to the standard Hygienic Laboratory method,[1] in which the Rawling strain of *B. typhosus* was the organism used. The vaccine contained 1,000,000,000 organisms per c. c. and was preserved with 0.3 per cent trikresol. The finished product was filled into 1-c. c. ampoules, which were sealed and stored at four different temperatures, viz, 5° C., refrigerator temperature; 10–15° C., cold-room temperature; 20–30° C., room temperature; and 37° C., incubator temperature. Rabbits were inoculated at stated periods in accordance with the Hygienic Laboratory method of testing typhoid vaccine,[1] the vaccine being administered subcutaneously in three doses, of ½ c. c., 1 c. c., and 1 c. c., at four or five day intervals and the rabbits bled about five days after the last injection. At each test three rabbits were always injected with each vaccine under test, and three control rabbits were injected with a Hygienic Laboratory vaccine of recent date, except in the tests made after three and six months' storage. Some of the rabbits died before immunization was completed.

The following protocols indicate the deterioration taking place during the time the vaccines were studied, as shown by the agglutination reactions of the serums of the vaccinated rabbits against a suspension of the Rawling strain. The culture was grown on agar slants or agar contained in Blake bottles, incubated 24 hours and the saline suspension of this growth made up to a turbidity corresponding to approximately 1,000 parts per million of silica in distilled water, making with the serum dilutions used a final dilution of 500 parts per million.

AGGLUTINATION AT BEGINNING OF TEST.

[4 indicates complete agglutination with supernatant fluid perfectly clear; 3, marked agglutination with supernatant fluid slightly turbid; 2, definite agglutination with supernatant fluid more turbid than in 3; 1, slight agglutination.]

	1/50	1/100	1/200	1/400	1/800	1/1,600[2]
Rabbit 1	4	3	2	2	2	1
Rabbit 2	4	4	3	2	2	1
Rabbit 3	4	3	3	2	2	2

[a] Manuscript submitted Nov. 1, 1919.

[1] McCoy, G. W., Hygienic Laboratory Bulletin No. 110.

[2] Final dilutions of serum.

3 MONTHS' STORAGE.

	1/50	1/100	1/200	1/400	1/800	1/1,600
5°.						
Rabbit 1	4	3	3	3	2	1
Rabbit 2	4	4	3	3	3	2
10–15°.						
Rabbit 1	4	4	3	2	1	1
Rabbit 2	4	3	3	2	1	1
Rabbit 3	4	3	2	1	1	1
20–30°.						
Rabbit 1	4	3	3	2	1	1
Rabbit 2	4	4	3	3	2	1
Rabbit 3	4	4	3	2	2	1
37°.						
Rabbit 1	4	4	4	3	2	1
Rabbit 2	4	4	3	3	2	1
Rabbit 3	4	4	4	3	2	2

6 MONTHS' STORAGE.

5°.						
Rabbit 1	4	4	4	3	2	1
Rabbit 2	4	4	4	4	4	2
Rabbit 3	4	4	4	3	2	1
10–15°.						
Rabbit 1	4	4	3	2	1	1
Rabbit 2	4	4	3	2	2	1
Rabbit 3	4	4	3	2	1	1
20–30°.						
Rabbit 1	4	4	3	2	1	0
Rabbit 2	4	4	3	1	0	0
Rabbit 3	4	4	3	2	1	0
37°.						
Rabbit 1	4	4	4	3	1	0
Rabbit 2	4	4	4	2	1	0

9 MONTHS' STORAGE.

5°.						
Rabbit 1	4	4	4	4	3	2
Rabbit 2	4	4	4	4	2	1
Rabbit 3	4	4	4	4	2	1
10–15°.						
Rabbit 1	4	4	4	4	3	3
Rabbit 2	4	4	4	4	3	3
20–30°.						
Rabbit 1	3	3	2	1	0	0
Rabbit 2	4	4	3	2	1	0
Rabbit 3	4	4	4	3	2	1
37°.						
Rabbit 1	2	1	1	0	0	0
Rabbit 2	4	4	3	2	1	0
CONTROL VACCINE.						
Rabbit 1	4	4	3	2	0	0
Rabbit 2	4	4	4	4	3	2
Rabbit 3	4	4	4	3	2	1

12 MONTHS' STORAGE.

	1/50	1/100	1/200	1/400	1/800	1/1,600
5°.						
Rabbit 1	4	3	2	2	1	0
Rabbit 2	4	3	2	1	1	0
Rabbit 3	4	2	1	0	0	0
10–15°.						
Rabbit 1	4	3	2	1	1	0
Rabbit 2	4	3	2	1	0	0
Rabbit 3	4	4	3	2	1	0
20–30°.						
Rabbit 1	3	3	2	1	0	0
Rabbit 2	2	1	1	0	0	0
Rabbit 3	4	3	2	1	0	0
37°.						
Rabbit 1	2	1	1	0	0	0
Rabbit 2	2	1	1	0	0	0
Rabbit 3	2	1	1	0	0	0
CONTROL VACCINE.						
Rabbit 1	4	4	3	2	1	0

15 MONTHS' STORAGE.

5°.						
Rabbit 1	4	3	2	2	1	0
Rabbit 2	4	3	2	2	1	0
10–15°.						
Rabbit 1	4	4	4	3	2	2
Rabbit 2	3	3	2	2	1	0
20–30°.						
Rabbit 1	4	3	2	2	1	0
Rabbit 2	4	3	2	2	0	0
Rabbit 3	4	3	2	1	1	0
37°.						
Rabbit 1	2	1	0	0	0	0
Rabbit 2	1	0	0	0	0	0
Rabbit 3	1	1	0	0	0	0
CONTROL VACCINE.						
Rabbit 1	4	3	3	2	0	0
Rabbit 2	4	3	2	2	1	0

18 MONTHS' STORAGE.

5°.						
Rabbit 1	4	3	2	1	0	0
Rabbit 2	4	3	3	3	2	0
10–15°.						
Rabbit 1	3	2	1	0	0	0
Rabbit 2	4	4	3	2	1	0
Rabbit 3	4	4	3	3	3	2
20–30°.						
Rabbit 1	4	4	3	1	0	0
Rabbit 2	4	3	3	3	2	0
Rabbit 3	3	3	2	1	0	0
37°.						
Rabbit 1	1	0	0	0	0	0
Rabbit 2	1	0	0	0	0	0
Rabbit 3	1	0	0	0	0	0
CONTROL VACCINE.						
Rabbit 1	4	4	3	3	3	2
Rabbit 2	4	4	4	3	2	1
Rabbit 3	4	3	2	2	1	0

24 MONTHS' STORAGE.

	1/50	1/100	1/200	1/400	1/800	1/1,600
5°.						
Rabbit 1	2	1	0	0	0	0
Rabbit 2	3	2	1	0	0	0
Rabbit 3	3	3	2	1	0	0
10–15°.						
Rabbit 1	4	3	1	1	0	0
20–30°.						
Rabbit 1	4	2	0	0	0	0
Rabbit 2	3	3	2	1	0	0
Rabbit 3	4	3	0	0	0	0
CONTROL VACCINE.						
Rabbit 1	4	4	4	3	1	0
Rabbit 2	3	2	1	0	0	0

31 MONTHS' STORAGE.

10–15°.						
Rabbit 1	4	2	1	0	0	0
Rabbit 2	4	3	1	0	0	0
20–30°.						
Rabbit 1	3	1	0	0	0	0
Rabbit 2	4	3	2	1	0	0
Rabbit 3	2	1	0	0	0	0
CONTROL VACCINE.						
Rabbit 1	4	3	3	3	1	0
Rabbit 2	4	4	4	3	3	0
Rabbit 3	4	3	3	3	2	0

32 MONTHS' STORAGE.

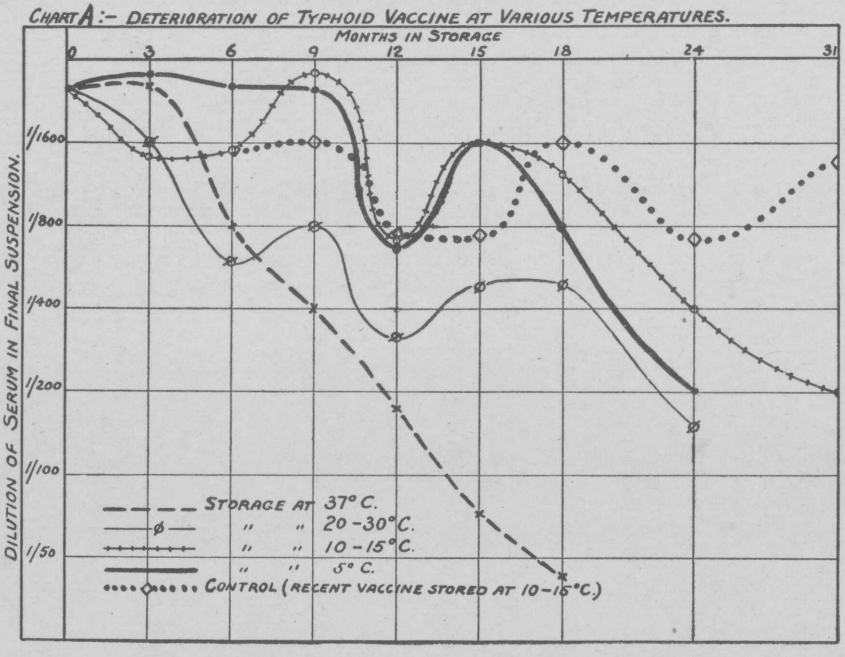
10–15°.						
Rabbit 1	4	3	3	1	0	0
Rabbit 2	4	4	2	0	0	0
CONTROL VACCINE.						
Rabbit 1	4	4	4	3	1	0
Rabbit 2	4	4	4	4	3	0
Rabbit 3	4	4	4	4	3	1

The graphs (Chart A) have been made by plotting points representing averages of the results obtained in the agglutination tests with the serums of each group of three rabbits against the suspensions of cultures. The approximate vanishing points of agglutination or those which by our method of reading would be recorded by 1+ are represented on the curve.

The curves as plotted from these results show that the highest temperatures are most detrimental to the vaccine. While the vaccine stored at 5° and 10–15° did not show any appreciable deterioration in 6 months, the vaccine stored at 37° showed a marked falling off in potency, which continued at a rapid rate, so that in 18 months this vaccine was practically without effect on rabbits. The vaccine stored at room temperature had deteriorated more than the vaccine

stored at the lower temperatures in 6 months and the curve continues below these two throughout the period of the test, though not falling off as abruptly as the curve representing the 37° storage.

In the case of the vaccines stored at 5° and 10–15° the effects of length of storage did not begin to be very apparent until after 18 months. By the time 24 months had elapsed the vaccine stored at 5° did not produce agglutinins in the rabbit serum which could be detected above a dilution of 1/200, and the one stored at 10–15° was of about the same strength, in contrast to a recently prepared vaccine which produced agglutinins, discernible in 1/800 dilutions of the serum. The experiment with the vaccine stored at 5° was discon-



tinued at the end of 24 months. The vaccine stored at 10–15°, at the end of 31 months, showed agglutinin production in the serum in a dilution of 1/200, and the test was repeated a month later and showed approximately the same result, the serum from the control vaccine showing agglutination between the dilutions 1/800 and 1/1,600 in both tests.

The results obtained indicate that the rapidity of deterioration is in direct proportion to the temperature above 15° C. The vaccine stored at 5° C. apparently deteriorated more rapidly than the one stored at 10–15°, but it is possible that if the serum from more rabbits had been available it would have been found that the difference

between the two curves would not have been as much as indicated by the diagram.

In the absence of more trustworthy tests for the determination of the potency of typhoid vaccine, we are compelled to rely on the determination of the agglutinin-producing properties as a measure of the probable potency of the vaccine and on the loss of this property for a measure of the loss of potency. The results of the work related indicate therefore that a storage temperature of not more than 15° C. is necessary to maintain typhoid vaccine at its maximum potency for the greatest length of time.

II. STANDARDIZATION OF GAS GANGRENE ANTITOXIN.

By Ida A. Bengtson, *Bacteriologist*, U. S. Public Health Service.

Antiserums for the various anaerobic organisms associated with gas gangrene have been used to some extent to prevent development of this complication of wounds, and, to a less extent, as a therapeutic measure. Though the exact value of these serums for prophylactic and curative purposes remains to be established it became necessary to undertake the studies in standardization which are reported here in order that the products used might be as potent and uniform as practicable.

A number of studies on the subject of the bacterial flora of gangrenous wounds were carried on during the World War of 1914–18, with the purpose of determining which organisms are the causative agents and what are the factors concerned in producing the symptoms. It has been a question whether the injurious effects produced are brought about through mechanical effects due to excessive amounts of gas or to increased acid produced as a result of the metabolic activities of the organisms, or whether true toxins formed by the organisms are concerned. As a result of the work done the conception of the etiology and of the nature of gas gangrene has been materially altered in recent years. While all of the aspects of the subject are not as yet clearly understood, it has been demonstrated that certain anaerobic organisms commonly found in the gangrenous wounds are toxin formers and that the toxins formed are important factors in the effects produced in gas gangrene. Work has thus been directed toward the production of antitoxic sera to be used for prophylaxis and treatment.

It has been shown that not only one species of organism but several or numerous species may be present in gangrenous wounds and investigations have been undertaken to determine which of these are the active toxin producers. The subject thus becomes a very complex one.

In France until recently the *Vibrion septique* of Pasteur was considered of primary importance in gaseous infections, and little importance was attached to any other of the anaerobic organisms described. It was only with the new interest aroused in the study of gas gangrene in 1914 that the organism *B. perfringens* was considered of special significance.

In Germany the bacillus of malignant oedema, described in 1881 by Koch, who considered it identical with Pasteur's *Vibrion septique*,

first received attention in connection with the etiology of gangrenous wounds. Though the organism of malignant oedema is usually connected with animal disease, it was considered for a long time in the light of the work of Koch, Pasteur, and Chauveau and Arloing that the human emphysematous gangrene and the malignant oedema of animals were one and the same disease and caused by the same organism, and until 1893 gangrenous septicemia of French authors and the German malignant oedema were practically synonymous terms for the affection. The identity of Pasteur's *Vibrion septique* and Koch's *B. oedematis maligni* was never definitely established, however, and later studies indicate that they are different organisms, or at least that many of the laboratory cultures now known as *B. oedematis maligni* are not the same as the *Vibrion septique* studied in connection with gas gangrene during the late war. This view is not, however, accepted by all workers.

The discovery of a new anaerobic organism in 1892 by Welch and Nuttall in the blood and organs of a cadaver eight hours after death from aortic aneurysm marked the beginning of a new epoch. The organism of Welch and Nuttall was named by them *B. aerogenes capsulatus* and has also been designated as *B. welchii* by other writers In 1893 Fraenkel isolated a similar organism from several cases of gaseous phlegmons which he called *B. phlegmones emphysematosæ*. He later acknowledged his organism to be identical with that of Welch, though it still is spoken of as Fraenkel's bacillus in German literature. In France it appears that the work of Welch was overlooked and Veillon and Zuber, 1898, described an organism identical with Welch's *B. aerogenes capsulatus* from several different pathological processes which they designated *B. perfringens*.[a]

In England during the period following 1892 and up to 1908 very little was published on the subject of gas gangrene and there are only a few scattered cases reported in which the bacillus of malignant oedema was considered the causal agent.

Von Hibler in 1899 published an extensive investigation of the anaerobic bacteria concerned in infections of man and animals and this work was revised and extended in 1908. A number of points are cleared up in the latter work and the whole subject of the relation

[a] The nomenclature of this organism is in a state of great confusion. In this country it has recently been almost universally known as *Bacillus welchii* and this name has also been used by English workers. The terms *Bacillus aerogenes capsulatus* Welch and Nuttall, 1892, and *Bacillus phlegmones emphysematosæ* Fraenkel, 1893, being trinomials are invalid. *Bacterium aerogenes* is valid for another organism and much confusion would result if the name *Bacillus aerogenes* were adopted. *Bacillus capsulatus* is also invalid on grounds of priority. *Bacillus emphysematosus* was used by Kruse in 1896 and *Bacterium emphysematosum* (Kruse) was adopted by Migula in 1900 for Fraenkel's organism, but Kruse includes *Bacillus aerogenes capsulatus* and Migula *Bacterium welchii* as different species. Other names have also been used for identical or closely related species, and in view of the uncertainty existing in the identification and nomenclature of these forms the name *Bacillus perfringens*, Veillon and Zuber, 1898, the first proposed binomial in use for this organism at the present time, has been adopted in this paper. These authors give a complete and accurate description of the organism.

of pathogenic anaerobes to disease established on a somewhat better basis than had hitherto been possible. On the authority of v. Hibler's work, v. Werdth recognizes two types of gaseous infections: (1) Malignant oedema, in which the organism concerned is *B. oedematis maligni* (bacillus X of Hibler), and (2) gas gangrene (Gasbrand), in which *B. perfringens* and numerous other anaerobes and some aerobes are concerned.

V. Hibler's classification of anaerobes distinguishes between the bacillus of Ghon and Sachs (Pasteur's *Vibrion septique*), which is a nonproteolytic organism, and his own No. X, *B. oedematis maligni*, which is a proteolytic organism. This is apparently a valid distinction, and it would appear therefore that the organism now recognized as *B. oedematis maligni* Koch is a different organism from Pasteur's *Vibrion septique*.

At the beginning of the war the study of the flora of war wounds was undertaken in both France and England. With the progress of the work, two facts stood out prominently: (1) That *numerous* anaerobic organisms are concerned in gas gangrene, and (2) that it is difficult to separate these organisms in pure culture and to determine which are of chief etiological importance. The work which has been accomplished serves to emphasize the fact that the problem is a very complex one and that we do not know yet the true nature of the infection. There is still much confusion in the identification of the organisms concerned, and the same organism is described under various names by different authors. The principal difficulty lies in the fact, as has been just stated, that the separation of anaerobes is a matter requiring great care, and it is probable that considerable amount of work has been done with mixed cultures. The isolation of one organism, *B. perfringens*, in pure culture has been definitely accomplished, and it is acknowledged by practically all workers that this organism is found most frequently of all the anaerobes in gangrenous wounds. As to the relative frequency of occurrence and identification of the other organisms concerned there is still some question.

The work in France has been carried on principally by Weinberg and Séguin, and Jouan of the Pasteur Institute and by Sacquépée. The first two authors studied the bacterial flora of 126 cases of gas gangrene and gaseous phlegmons and as the result of their work state that 12 species of anaerobes are concerned in gas gangrene. Eight of these had been previously recognized and four are new species. The three organisms to which they assign the principal rôle are *B. perfringens*, *B. oedematiens*, and *Vibrion septique*. *B. oedematiens* is a new species isolated by them which produces a very potent toxin, and *Vibrion septique* is apparently the same organism as that originally described by Pasteur. In addition to these,

B. sporogenes and *B. fallax* were present frequently; in fact, these occurred more often than the *Vibrion septique*, but the authors consider them of less importance from the pathogenic viewpoint than the three mentioned. *B. sporogenes* was next in frequency of occurrence to *B. perfringens*, but it produces a less potent toxin. This organism, which is motile, is characterized by its active proteolytic power and is usually associated with the putrid forms of gas gangrene. It has been confused with Pasteur's *Vibrion septique*.

B. fallax, a new species isolated by Weinberg, resembles *B. perfringens*. This author states that the organism produces a feeble toxin, 1–2 c. c. injected intravenously causing the death of 300–500 gram guinea pigs.

In addition to these five organisms a number of others of less frequent occurrence, including *B. putrificus* (Bienstock), *B. tertius* (Henry), *B. bifermentans* (Tissier and Martelly), *B. aerofoetidus* (Weinberg and Séguin), and *B. histolyticus* (Weinberg and Séguin), are considered as concerned in gas gangrene by Weinberg and Séguin. Sacquépée has described an organism occurring in gas gangrene which was first designated by him as *B. d'Œdème gazeuse malin* and later as *B. bellonensis;* in some of the descriptions this appears closely related to *B. oedematiens*, but the exact relationship of the organisms is somewhat obscure.

Much of the work done in Great Britain has been concerned with the identification of the various organisms present in gas gangrene and on studies of the biochemical properties of these organisms. The first report of this work was made by Miss Robertson of the Lister Institute who examined wound material and isolated as the three most frequently occurring organisms, *B. perfringens, B. odematis maligni* and an organism closely resembling v. Hibler's bacillus No. IX. Weinberg questions the identification of *B. oedematis maligni* in this study, and Henry in a later study classifies this group of organisms which are motile and proteolytic under the title *B. sporogenes.* The third group described by Miss Robertson became *B. tertius* of Henry, so named because it was third in frequency of occurrence. This organism was found on only one or two occasions by Weinberg and Séguin, and is considered of minor importance by them as it was not found to be pathogenic for guinea pigs. On the other hand, the organism *B. oedematiens,* which Weinberg and Séguin placed as third in frequency of occurrence in gangrenous wounds examined by them, was not isolated by Henry, but was later isolated by another English worker, Dalyell.

As the matter stands now it appears therefore that *B. perfringens* is most frequently present in war wounds and *B. sporogenes* second. *B. oedematiens* has been found to be third in frequency of occurrence, according to Weinberg and Séguin, and produces the most powerful

toxin of any of the anaerobes concerned in gas gangrene. *Vibiour septique* also occurs frequently.

The results of the investigations of the French workers on the etiology of gas gangrene have in the main been accepted in England and in this country. With the fact established that the organism *B. perfringens* is present in the great majority of gangrenous wounds, and that *B. oedematiens* and *Vibrion septique* are also present in a certain percentage of such wounds, and that all three are toxin producers, attention has been directed toward the preparation of antitoxins to be used in the treatment of gangrenous wounds.

An antitoxin against *B. perfringens* was successfully prepared by Bull and Pritchett at the Rockefeller Institute in 1917; a less effective antiserum had previously been reported by Klose. An antitoxin against *Vibrion septique* was produced in France in 1915 by Nicolle, Cesari, and Raphael, which was feebly protective. Later in the same year more potent toxins from the latter organism were produced by Jouan and by Raphael and Frasey and more effective antitoxins were obtained. Weinberg and Séguin isolated the new species *B. oedematiens* in 1915 and later demonstrated a soluble toxin against which animals could be immunized.

The work on the production of the antitoxin for use in gas gangrene cases was begun in this country about June 1, 1918, at which time it was recommended by the board of the Central Medical Laboratory of the American Expeditionary Forces in France that the production of combined antitetanus and antigas-gangrene serum be undertaken by the manufacturing establishments in this country for use among the American troops in France.

It was the purpose in the manufacture of this serum to use horses which had previously been injected with tetanus toxin, in order that a composite serum against the most important organisms concerned in gas gangrene, as well as against the tetanus organism, might be produced as rapidly as possible.

Injections of tetanus horses with the filtrates of toxicogenic cultures of *B. perfringens* were begun at once at several of the manufacturing establishments and the first serum sent in for test was received at the Hygenic Laboratory in August of 1918.

The method of testing adopted is similar to that used in the Hygienic Laboratory method of testing tetanus and diphtheria antitoxins, with necessary modifications. Much of the basis for this work is related in the Hygienic Laboratory bulletins Nos. 21 and 43.

Toxin production by *B. perfringens* and work on the standardization of this antitoxin was first undertaken and it was proposed to follow this by investigation of the other two organisms, *Vibrion septique* and *B. oedematiens.*

TOXIN OF B. PERFRINGENS (B. WELCHII).

The first work necessary was to obtain a toxin for use in testing the strength of the antitoxin. Advantage was taken of the published work of Bull and Prichett and DeKruif and their personal suggestions. Considerable experimentation was necessary to ascertain the best conditions for producing a good toxin. The method described in the following pages was the one used in making several of the best toxins.

Medium.—The medium used in most of the work was that used first by DeKruif, Adams, and Ireland, which was a modification of Bull's original medium. In place of the fresh pigeon muscle used by Bull, DeKruif experimented with macerated veal and found that practically as good a toxin could be obtained with this, with the additional advantage that the medium could be sterilized after the addition of the meat. The medium used at the Hygienic Laboratory consisted of beef infusion broth and chopped fresh veal in the proportion of 200 grams of the veal to 300 c. c. of the broth. Sterile glucose solution was added after sterilization in the proportion of 0.2 per cent of the total volume. The medium used in most of the work was contained in 500 c. c. Kjeldahl flasks, which on account of the small surface exposure are well adapted to the growth of anaerobic organisms.

The disadvantage of using a broth-meat medium lies in the fact that it is difficult to adjust the reaction to the desired end point, though it is probably true that the range favorable for toxin production is rather extended. Veal in the process of autoclaving produces a large amount of acid and allowance must be made for any additional heating after sterilization. Different lots of veal vary in regard to acid production, and it is difficult to control absolutely the factor of heat so that the desired final reaction may be obtained. *B. perfringens* is not as sensitive to acid as *B. diphtheriae*, however, and toxin is produced in media of much higher acidity. DeKruif recommends an initial reaction of +0.5 per cent to phenolphthalein. The end reaction favorable for the best toxin production was studied by Bull and Pritchett, who found that good toxin was produced in media, which after 24 hours incubation titrated +2 to 4 per cent acid to phenolphthalein, but when the reaction reached a point as high as +6.8 per cent the toxin was very weak.

The method used in the adjustment of the reaction in the present work when the best toxins were obtained was first to heat one flask of the batch of medium for the total length of time which was to be used for sterilization and subsequent heating, then to titrate for acid

production and calculate the amount of alkali necessary to bring the final reaction somewhere near the desired point.

The rough preliminary adjustment of the medium was made by titration against phenolphthalein, but at various stages records were also made of the H-ion concentration as determined by the Sörensen colorimetric method. In order to obtain a medium which was favorable for good toxin production, as determined by experience, it was usually found necessary to add sufficient alkali to the medium to bring the reaction to a point represented by a *p*H value of 9 to 9.2 (which is considerably on the alkaline side of the neutral point as measured by phenolphthalein) in order to neutralize the acid produced in the process of sterilization. The purpose was to produce a medium which after the final sterilization and subsequent heating would approximate a reaction of *p*H 7.4. It was found that the individual flasks of medium varied to a considerable extent in reaction, usually ranging from *p*H 7 to 7.5. In the last lot of toxin made, which was one of the best, acidity went even higher than this, the different flasks varying from 6.7 to 6.9.

The flasks were inoculated with 10–15 c. c. of a 24-hour growth of culture. The culture had been passed through two or three pigeons before use in the inoculation of the flasks. The pigeons were usually injected in the morning with a sufficiently large amount of the culture to kill before the end of the afternoon, and the infected muscle tissue was planted into glucose broth fermentation tubes and incubated overnight. After the last pigeon passage a number of tubes to be used for planting were inoculated with the infected muscle and incubated for about 10 hours or overnight. Smears were made to determine purity of the culture, and the flasks were planted with the culture from the various tubes.

The flasks were usually heated in the Arnold sterilizer for a period of one-half hour before using and planted while still warm. Under these conditions growth was apparent within two or three hours, as evidenced by the vigorous production of gas. The incubation period was 21–24 hours.

Centrifugation was found necessary before attempting to filter if a potent toxin was desired. This was continued for three-fourths of an hour, at a speed of about 1,500 revolutions per minute.

The filtration was accomplished by means of Berkefeld N-filters. Considerable difficulty was experienced in the beginning of the work in carrying out the filtration, and apparently the strength of the various toxins obtained was in a great measure dependent on the rapidity of filtration. As a rule, when filtration was rapid a fairly good toxin was obtained, though there were exceptions to this rule.

Some fairly potent toxins, as *Perfringens* toxins go, were obtained by the above method. The test dose for a 350-gram pigeon, as measured by the standard set by the Hygienic Laboratory, immediately after filtration was in the neighborhood of 2 c. c. and the minimum lethal dose 0.12 to 0.2 c. c. In the beginning of the work some toxins were used which had a test dose of over 3 c. c., but in the later part of the work the preliminary tests were always made with doses of 2 c. c. and 3 c. c., and toxins which did not kill on the latter test were not used further.

On the day following filtration the toxin was filled into drawn-out glass tubes which had a small surface exposure; these were sealed and stored at ice-box temperature. This method was adopted for convenience in use and also with the idea that deterioration would be less in the sealed ampoules than in a large container.

Antitoxin.—The antitoxin used in developing a standard was one furnished by the Rockefeller Institute and prepared by Maj. Bull.

STANDARDIZATION OF THE ANTITOXIN OF B. PERFRINGENS (B. WELCHII).

The Hygienic Laboratory standard was established as follows:

The unit shall be 1 c. c. of the standard serum which is kept in cold storage. To estimate the potency of a commercial antitoxin, the test toxin shall first be standardized by inoculating pigeons intramuscularly with 1/100 unit of standard serum mixed with varying amounts of toxin to determine the smallest dose of toxin which will overcome this amount of serum and kill the pigeon within 24 hours. This dose of toxin, called the "test dose," is usually somewhat greater than 10 minimal lethal doses. The test dose of toxin is then to be mixed with varying amounts of the serum to be tested and injected into a second series of pigeons; that amount of serum which gives protection for 24 hours against the test dose of toxin shall be considered to contain 1/100 unit. The serum-toxin mixtures are incubated 45 minutes at 37° C. before injection. Pigeons should weigh preferably between 325 and 375 grams, but the doses of toxin and antitoxin shall be proportioned to the weight, 350 grams being taken as the standard weight.

Provision for deterioration of serums produced by the manufacturers was made by requiring a 25 per cent excess in unitage over the number of units stated on the label.

This unit is of the same nature as the American units of tetanus and diphtheria antitoxins, that is, it is the antitoxin contained in an arbitrary amount of serum, and standardization of an unknown antitoxin is effected by comparing the respective neutralizing powers against a dose of toxin containing a number of minimal lethal doses and not against one minimal lethal dose. There was no reason to suppose that the toxin produced by *B. perfringens* differed from that of diphtheria or tetanus in the matter of containing a variable proportion of toxoids, or nontoxic groups, which still had the power

of combining with antitoxin; and therefore as is true in the case of the latter antitoxins, a test of the antitoxin against the combining dose of the toxin should give a more correct test of potency of the serum than a test against one minimal lethal dose.

The neutralizing power of the Perfringens unit as established falls somewhere near that of the tetanus unit, so that the method of stating the potency of the composite serum gives a fair idea of the comparative strength of the two antitoxins. The American unit of tetanus antitoxin[1] neutralizes somewhat less than 1,000 minimal lethal doses of tetanus toxin, since the test dose of tetanus toxin is that amount which is almost neutralized by one-tenth of a unit of the standard antitoxin, and contains about 100 minimal lethal doses. In the case of Perfringens, one one-hundredth of the antitoxin unit just fails to neutralize 10 or somewhat more minimal lethal doses of Perfringens toxin, depending on the composition of the toxin; and therefore the unit neutralizes about 1,000 minimal lethal doses. However, none of the Perfringens antitoxins produced approached in neutralizing power per cubic centimeter the tetanus antitoxins. For example, in a sample containing 15 c. c. of serum there might be 1,500 units of tetanus antitoxin, or 1 unit in one one-hundredth of a c. c., while in the same sample there might be only 15 units of Perfringens toxin, or 1 unit in 1 c. c. One c. c. of the tetanus antitoxin therefore neutralizes about 100,000 minimal lethal doses of tetanus toxin and 1 c. c. of the Perfringens antitoxin neutralizes only about 1,000 minimal lethal doses of the corresponding toxin; in other words, the volume of the Perfringens antitoxin required to neutralize a certain number of minimal lethal doses is 100 times as large as the volume of tetanus antitoxin necessary to neutralize the same number of tetanus minimal lethal doses. It is thus evident that the method adopted of stating the potency gives a fair idea of the comparative strengths of the two antitoxins.

It may be remarked here that it was found very difficult for manufacturers to produce Perfringens antitoxin containing more than 1 unit per c. c., though some of the latest specimens received showed as much as 2 units per c. c.

The following protocol shows the method of testing a Perfringens antitoxin for potency.

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[1] Regulations for the sale of viruses, serums, toxins, and analogous products in the District of Columbia and in Interstate Traffic, Sect. 72, Washington, 1919.

No. of pigeon.	Weight.	Toxin.			Antitoxin.					Saline.	Hour injected.	Hour died.	Hours survived.	Necropsy.
		No.	Dose per 350 grams.	Actual dose.	No. of antitoxin.	Dose per 350 grams.	Actual dose.	Dilution.	Amount of dilution.					
	Gms.		*c.c.*	*c.c.*		*c.c.*	*c.c.*		*c.c.*	*c.c.*	*P.m.*	*A.m.*		
583	380	14B	2.6	2.82	Antitoxin X.	0.0135	0.0146	1+99	1.46	0.72	4.30		(1)	
584	370	14B	2.6	2.74	.....do.....	.012	.0127	1+99	1.27	.99	4.30		(1)	
585	360	14B	2.6	2.68	.....do.....	.012	.0123	1+99	1.23	1.09	4.30		(1)	
586	330	14B	2.6	2.45	.....do.....	.01	.0094	1+99	.94	1.61	4.30	4 *P.m.*	11½	Typical appearance (see below).
587	365	14B	2.6	2.71	Standard antitoxin.	.01	.0104	1+99	1.04	1.24	4.30	10	5½	Typical appearance.
588	330	14B	2.6	2.45	.....do.....	.01	.0094	1+99	.94	1.61	4.30	10	5½	Do.
589	320	14B	2.6	2.38	.....do.....	.01	.0091	1+99	.91	1.71	4.30	9	4½	Do.

[1] Survived.

Antitoxin X, therefore, contains 1/100 unit in slightly less than 0.012 c. c. and contains 10 units, the minimum routine human dose, in 12 c. c., with some excess.

The pigeons which do not survive usually die within the first 24 hours, and this length of time is taken as the limit for the test. A necropsy is made on all pigeons dying within this time, though the pigeons surviving are always examined for swelling and discoloration, and it is possible to judge from the extent of the lesions something as to the strength of the antitoxin. The lesions include swelling and necrosis of the muscle tissue, and hemorrhagic gelatinous exudate which is usually very abundant in the subcutaneous tissue of the groin on both the inoculated and the uninoculated sides. The exudate may be found under the breastbone also in severe cases. The internal organs show no characteristic lesions.

DETERIORATION OF TOXIN.

In the work of testing the antitoxins it was found necessary to use the toxins in the liquid form soon after they were obtained, since the time was too short to permit using a toxin which had been stored and studied first as to deterioration. In addition to the routine tests on the antitoxins, a number of tests were made as time permitted to ascertain whether the same factors influence deterioration as applied to diphtheria toxin, and thus to determine the best conditions for storing the toxin in order to keep deterioration at a minimum. In the case of diphtheria toxin, the usual procedure followed is to use a seasoned toxin; that is, one which has passed through the preliminary stages of deterioration, which may be six months or more. Rosenau [2] showed that toxin suffers a gradual loss of potency during this period and then reaches a comparatively stable condition which continues for a long period, during which time changes are very slight.

It soon became evident in the work on Perfringens toxin that this toxin has considerable stability, or at least the rate of change was gradual enough so that it could be used without fear of its suddenly losing

[2] Hygienic Laboratory Bulletin, No. 21, 1912.

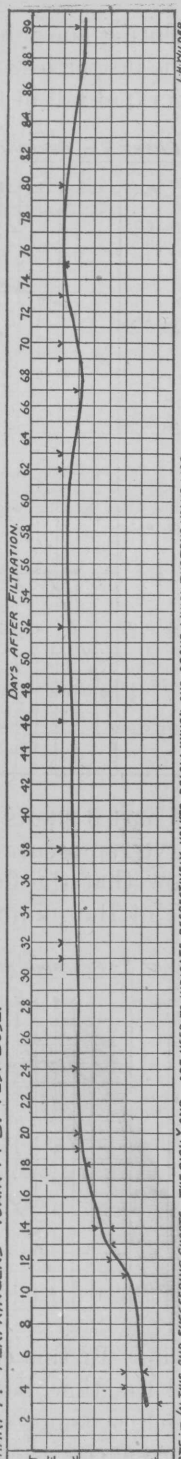
in potency. In this respect therefore it resembles diphtheria toxin more closely than tetanus toxin, which is very unstable in the liquid form.

The length of time necessary for the toxin to reach a stable condition appeared to be shorter than is the case with diphtheria toxin. In determining this change, the tests were always made against the test dose of toxin though in some cases tests of the change in minimal lethal doses were also made in order to determine the comparative loss in toxicity and in combining power.

The accompanying diagrams indicate the change taking place in several toxins during periods covering one to three months. The toxins as before stated were contained in drawn out and sealed glass tubes which left only a small amount of surface exposed. The ampoules contained between 15–20 c. c. of toxin and the temperature of storage was 5° C. It is probable that for a very accurate study of deterioration effects, storage in large containers, from which samples could have been removed under aseptic conditions from time to time, would have proved more satisfactory as the change would have been uniform throughout the whole bulk, whereas with the small containers the possibility exists that the rate of change in different ampoules may have varied owing to slight differences in conditions. Under the conditions obtaining and for the reason that it was necessary to carry out the tests on antitoxins needed for emergency military purposes before all the experimental work could be undertaken to determine these points, it was thought advisable to use small ampoules as containers for the fluid toxin and to titrate for potency at frequent intervals and just previously to testing the antitoxins which were received for tests.

Chart 1 shows the loss of combining power in toxin 14B which when first used had a test dose of somewhat less than 2.2 c. c. and in 90 days showed a test dose of less than 2.5 c. c.

The curves representing the change in the test dose and the minimal lethal dose of toxin 6 (chart 2) in a period covering 36 days shows how the test dose is a more nearly constant quantity than the minimal lethal dose, and therefore a more satisfactory measure to be used in testing the strengths of antitoxins. The rapid change in the curve rep-

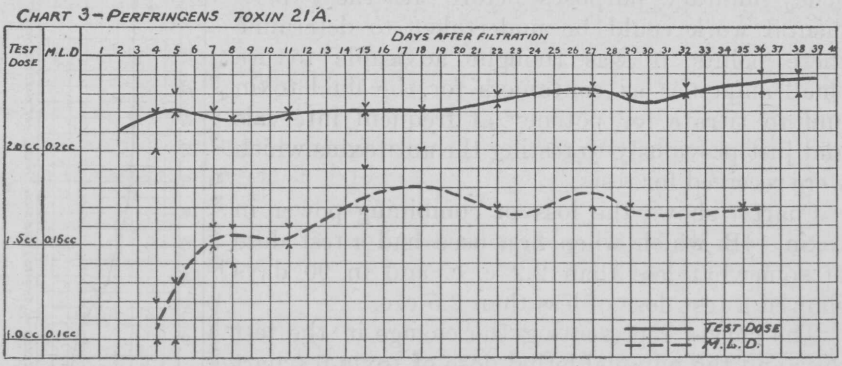


resenting the minimal lethal dose indicates a rather rapid fall in the toxic properties and the more gradual change in the test dose shows a less rapid fall in the combining power of the toxin with the antitoxin.

CHART 2 – PERFRINGENS TOXIN 6.

Chart 3 shows the results obtained in the case of toxin 21A. The curve representing the changes occurring in the minimal lethal dose show that the fluctuation of this measure would make difficult the use of the minimal lethal dose in testing the strength of the anti-

CHART 3 – PERFRINGENS TOXIN 21A.

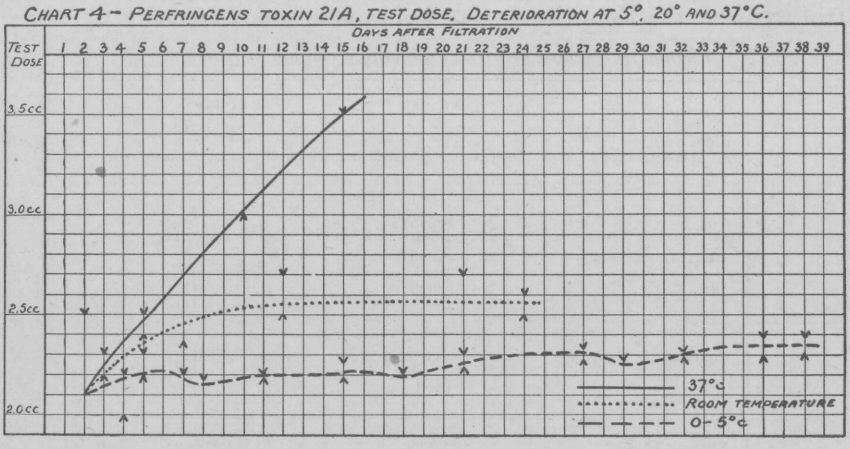


toxin. In this case the intervals between test doses were smaller and the time between tests was shorter than in the case of toxin 6, which accounts for a certain part of the irregularity. The curve representing the test dose at corresponding intervals of time as before

shows that the loss of combining power is proportionately less than the loss of toxic properties.

The deterioration is probably influenced by several factors, including light, temperature, reaction, oxygen tension. The effect of temperature on deterioration of the toxin is shown in the accompanying diagram (Chart 4). The test dose was determined for the three temperatures, 5° C. room temperature, and incubator temperature. The deterioration at warm-room temperature as far as tested was very rapid, as is shown by the curve rising abruptly at an angle of about 45°. At room temperature the loss was less rapid and after about 10 days the toxin apparently became quite stable. The deterioration at 5° C. was very gradual compared with that at the other two temperatures, and after 40 days the strength apparently was at

CHART 4- PERFRINGENS TOXIN 21A, TEST DOSE. DETERIORATION AT 5°, 20° AND 37°C.



about the same point as at 22 days. A low temperature is therefore indicated for storage of the toxin.

The effect of light on deterioration was tested by exposing vials of the toxin to direct sunlight. An exposure of three and one-half hours had no immediate effect on the toxin, as the pigeons inoculated with the exposed toxin died on the same dose as the control toxin. An exposure of seven hours to sunlight had the effect of increasing the test dose from 2.25 c. c. to 2.55 c. c.

The following protocol indicates this:

	Dose of toxin.	Result.
AMPOULE UNEXPOSED.	c. c.	
Pigeon 1	2.25	Died, 9 hours.
Pigeon 2	2.3	Died, 16 hours.
Pigeon 3	2.35	Died, 15 hours.
Pigeon 4	2.4	Died, 16 hours.
AMPOULE EXPOSED TO SUNLIGHT 7 HOURS.		
Pigeon 5	2.25	Survived.
Pigeon 6	2.35	Do.
Pigeon 7	2.45	Do.
Pigeon 8	2.55	Died, 9 hours.

In these tests the control ampoules were kept at the usual temperature of storage (5° C.). Since the period of exposure to sunlight was of such short duration, it is probable that controls kept at the same temperature but not exposed to sunlight would not have shown any appreciable change.

The effect of the addition of acid and alkali as regards the keeping qualities of the toxin was tested by adding varying amounts of hydrochloric acid and sodium hydroxide to the toxin and storing at the temperature 5° C. The method used was to add measured amounts of sterile N/1 or N/10 HCl and NaOH to 10 c. c. amounts of the toxin, due allowance being made for the increase in volume in making the test. The reaction of the toxin before the addition of acid and alkali was represented by a *p*H value of 6.8. The accompanying table shows the results obtained. The test dose of the control toxin at the time these tests were made was 2.25–2.3 c. c.

Change in test dose of toxin following a change in reaction.

Amount of N/1 HCl added to 10 mil of toxin.	*p*H.	3 days.		11 days.	
		Dose.	Result.	Dose.	Result.
		c. c.		c. c.	
0.01	6.8	2.25	Survived		
		2.5	Died	2.5	Survived.
.1	6.4	2.25	Survived		
		2.5	Died		
.15	6.2	2.25	Survived	2.3	Do.
		2.5	Died	2.5	Do.
Controls		2.25	Survived	2.25	Died.
		2.25	do	2.3	Do.
		2.3	Died		

Amount of N/1 NaOH added to 10 mil of toxin.	*p*H.	3 days.		11 days.	
		Dose.	Result.	Dose.	Result.
		c. c.		c. c.	
0.01	7.0	2.25	Died	2.3	Survived.
		2.5	do	2.5	Died.
.1	7.3	2.25	do	0.15 N/1 NaOH 2.3	Survived.
		2.5	do	2.5	Do.
.2	7.5	2.25	Survived		
		2.5	do	2.5	Do.
Controls		2.25	Survived	2.25	Died.
		2.25	do	2.3	Do.
		2.3	Died		

In the case of the acid it is seen that all the pigeons on the dose 2.25 c. c. survived, while all on 2.5 c. c. died after the acid had been allowed to act three days. After 11 days the toxin had evidently deteriorated to such an extent that the pigeons survived on both doses 2.3 and 2.5 c. c.

In the case of the alkali, the addition of up to 0.1 c. c. of N/1 NaOH to 10 c. c. of toxin had no effect for three days; all of the pigeons

inoculated with 2.25 and 2.5 c. c. died. An amount as high as 0.2 c. c. of N/1NaOH seemed to have changed the toxin to such an extent that neither of the pigeons inoculated with 2.25 or 2.5 c. c. died. After 11 days all of the pigeons inoculated with 2.3 c. c. of the toxin survived and in two cases the pigeons on 2.5 c. c. survived. While the above experiments indicated that a considerable change in the reaction is necessary to cause marked deterioration, it is probable that the cumulative effects of small changes may be the same after longer periods of time and that therefore the reaction of the glass container may influence deterioration of the toxin.

It is thus apparent that the fluid toxin of *B. perfringens* deteriorates to a certain extent just as other fluid toxins depending on varying conditions of heat, light, reaction, and other unknown factors, but that if kept under the most favorable conditions it is reasonably stable.

A precipitated toxin was prepared early in the work and several other attempts were made to obtain a considerable amount of the dry toxin, but in all cases the yield of toxin in proportion to the volume of fluid was very small. The usual method of precipitating by saturating with ammonium sulphate crystals was the method employed. The toxin was then dried in vacuo, and stored in vacuo in a Novy jar at a temperature of 10–15° C.

Protocol indicating the results of tests made on a dry toxin against the same antitoxin at intervals of about 10 months.

	Pigeon.	Weight.	Toxin test dose.				Antitoxin.					Hours survived.
			No. of toxin.	Dose per 100 grams.	Actual dose.	Amount of dilution.	No. of antitoxin.	Dose per 100 grams.	Actual dose.	Dilution.	Amount of dilution.	
		Gms		*Gms.*	*Gms.*	*c. c.*		*c. c.*	*c. c.*		*c. c.*	
May 31, 1918..	1a	405	3	0.002	0.0081	0.24	Antitoxin 5.	0.0075	0.030	1+29	0.9	Survived.
	2a	360	3	.003	.0108	.32	.....do.....	.0075	.027	1+29	.81	Do.
	3a	350	3	.005	.0175	.52	.....do.....	.0075	.026	1+29	.78	Do.
	4a	325	3	.007	.0227	.68	.....do.....	.0075	.024	1+29	.72	Do.
	5a	320	3	.010	.0320	.96	.....do.....	.0075	.024	1+29	.72	4½.
Mar. 21, 1919..	1119	355	3	.008	.0284	.85	.....do.....	.0075	.0264	1+29	.79	Survived.
	1120	340	3	.010	.034	1.02	.....do.....	.0075	.0252	1+29	.76	3.
	1121	325	3	.010	.0325	.98	.....do.....	.0075	.0242	1+29	.73	4.
	1122	320	3	.012	.0384	1.15	.....do.....	.0075	.0238	1+29	.81	4.
	1123	315	3	.012	.0378	1.03	.....do.....	.0075	.0234	1+29	.70	4.

	Pigeon.	Weight.	Minimal lethal dose.				Hours survived.
			No. of toxin.	Dose per 100 grams.	Actual dose.	Amount of dilution.	
		Grams.		*Grams.*	*Grams.*	*c. c.*	
May 31, 1918.....	11a	380	3	0.0002	0.00076	0.23	29½.
	12a	375	3	.0003	.00112	.34	13½.
	13a	340	3	.0005	.0017	.51	11½.
	14a	340	3	.0007	.0024	.72	9½.
	15a	310	3	.0010	.0031	.93	11½.
Mar. 22–23, 1919.....	1125	370	3	.0002	.00074	.22	3.
	1131	300	3	.0002	.0006	.18	Survived.
	1132	370	3	.0003	.0011	.33	17.
	1133	295	3	.0003	.00085	.255	12.

The test dose in the first experiment against the amount of antitoxin used lies between 0.025 and 0.035 gram of the dried toxin, and in the second test between 0.028 and 0.035 gram for 350 grams weight of pigeon. The minimal lethal dose in the first test was between 0.0007 and 0.00105 gram for 350 gram or very close to the lower limit, indicated by the fact that the pigeon died after the 24 hour limit. In the second test, the minimal lethal dose was very close to 0.0007 gram since one pigeon died on this dose and another survived. These tests though limited in number indicate that the dried toxin, like tetanus toxin, is a very stable product.

TOXIN AND ANTITOXIN OF VIBRION SEPTIQUE.

The culture used by the Hygienic Laboratory in the work on the standardization of *Vibrion septique* antitoxin and which was distributed to the manufacturing concerns was one obtained from Jouan of the Pasteur Institute, and is said to correspond to Pasteur's original *Vibrion septique*.

In its cultural reactions this organism agrees with Weinberg and Séguin's description. It was found to be quite distinct as regards cultural behavior from two cultures of *B. oedematis maligni* in the collection at the Hygienic Laboratory and also distinct from *B. oedematis maligni* as described by v. Hibler. Morphologically this organism is more slender than *B. perfringens* and forms spores readily. The organism is gram-positive, is motile and somewhat pleomorphic, presenting certain peculiar forms described as citron forms which are considered characteristic of the organisms in smears from wound material. Long filamentous forms may be obtained from the liver of guinea pigs inoculated with the culture.

The organism is nonproteolytic, failing to digest casein, blood serum, or minced meat. This is in marked contrast to the behavior exhibited by the cultures of *B. oedematis maligni* which were tested in the same media. Gas was formed in glucose broth, but it is not as active in fermenting this sugar as is *B. perfringens*. The organism produces a septicemia in guinea pigs and the culture can easily be isolated from the heart blood of an animal which has been injected subcutaneously. In this respect it differs from *B. perfringens*, which can not usually be obtained from the blood but must be isolated from the muscle tissue into which the culture was inoculated.

TOXIN.

The medium recommended by Jouan and also by Raphael and Frasey, who obtained potent toxins, was Martin's peptone glucose broth, in which Martin's peptone freshly made from pig's stomach subjected to peptic digestion is used. In this country a satisfactory toxin was produced by the use of a 0.2 per cent glucose veal broth

containing 10 per cent of sterile horse serum and this medium was used in the work carried on at the Hygienic Laboratory.

Several satisfactory toxins were obtained with somewhat less difficulty than was encountered in the case of the Perfringens toxin. The toxins were tested on pigeons, rabbits, and guinea pigs and in accordance with the methods of the French investigators injections were made intravenously. De Kruif [3] recommends small guinea pigs about 200 grams weight as satisfactory test animals, the injections to be made into the jugular vein. This method was used in part of the work, but rabbits proved to be more satisfactory on account of the greater ease in making the inoculation into the ear vein, and it was found that these animals are about as susceptible as guinea pigs, weight for weight.

The animals succumb in as short a space of time as five or ten minutes if a sufficiently large dose of toxin is injected, which fact raises the question whether the substance producing the injurious effects is a true toxin. A serum which neutralizes the effects of this substance has been produced, however, and it has also been shown that a longer period intervenes before death if inoculations are made by the subcutaneous route.

ANTITOXIN.

The only sample of antitoxin against Vibrion septique which was received at the Hygienic Laboratory was one obtained from the Pasteur Institute and this was to have been used as a standard serum in testing of serums received from manufacturers.

Standardization.—The French method for testing the potency of anti-*Vibrion septique* serums was used in comparing several toxins. In accordance with the French standard, 1/1,000 c. c. of the antitoxin should neutralize two fatal doses of the toxin after 30 minutes incubation of the mixture at room temperature.

The following protocols indicate results obtained with two lots of toxin tested for the minimal lethal dose and against the Pasteur Institute antitoxin:

Toxin 2B.

	Weight.	Toxin.			Antitoxin.			Result.
		No. of toxin.	Dose per 1,000 grams.	Actual dose.	Dose per 1,000 grams.	Dilution.	Actual dose.	
	Grams.		*c. c.*	*c. c.*	*c. c.*		*c. c.*	
Rabbit 1	1,230	2B	1.0	1.23	.....	.....	.....	Died in 2 minutes.
Rabbit 2	1,220	2B	.9	1.10	.....	.....	.....	Died in 6 minutes.
Rabbit 3	1,220	2B	.8	.98	.....	.....	.....	Survived.
Rabbit 4	1,400	2B	1.8	2.52	1/1,000	1/500	[a] 0.7	Survived 6 days.

[a] The test should have been carried out according to the French standard by using 0.5 c. c. of the dilution 1/500. In this case the dose of antitoxin was inadvertently made 0.7 c. c. (i. e. 0.5 c. c. per 1,000 grams).

[3] Personal communication.

After 8½ months storage this toxin had deteriorated to the extent that 1.5 mil per 1,000 grams failed to kill rabbits in less than five hours.

	Weight.	No. of toxin.	Dose per 1,000 grams.	Actual dose.	Length of time survived.
	Grams.		*c. c.*	*c. c.*	
Rabbit 1	1,350	2B	1.1	1.5	2 hours.
Rabbit 2	1,775	2B	1.2	2.13	4 hours.
Rabbit 3	1,650	2B	1.3	2.15	17½ hours
Rabbit 4	1,600	2B	1.4	2.24	19 hours.
Rabbit 5	1,820	2B	1.5	2.73	5 hours.

Toxin 3B (after 6 months' storage).

	Weight.	No. of Toxin.	Dose per 1,000 grams.	Actual dose.	Length of time survived.
			c. c.	*c. c.*	
Rabbit 1	1,080	3B	0.8	0.86	Survived.
Rabbit 2	1,190	3B	.9	1.07	12 hours.
Rabbit 3	1,270	3B	1.0	1.27	3 hours.
Rabbit 4	1,290	3B	1.1	1.41	5 hours.
Rabbit 5	1,340	3B	1.2	1.61	8 minutes.

	Weight.	Toxin.			Antitoxin.			Result.
		No. of toxin.	Dose per 1,000 grams.	Actual dose.	Dose per 1,000 grams.	Dilution.	Actual dose.	
			c. c.	*c. c.*	*c. c.*		*c. c.*	
Rabbit 6	1,220	3B	1.8	2.2	1/1,000	1/500	0.5	Died in 1½ hours.
Rabbit 7	1,270	3B	2.4	3.0	1/1,000	1/500	.5	Died in 9 minutes.

The amount of antitoxin used in the last test, 1/1,000 c. c., was insufficient to neutralize two fatal doses of the toxin, both twice the amount of toxin which killed in less than 10 minutes (1.2 c. c.) and twice the amount which killed in about 12 hours having been used.

B. OEDEMATIENS.

A culture received through Maj. Bull from Weinberg of the Pasteur Institute was used in several attempts to produce toxin, but no very satisfactory toxin was produced. Weinberg and Séguin state in their protocols that 1/50–1/100 c. c. of toxin inoculated subcutaneously was sufficient to kill guinea pigs in two to three days.

No toxin was obtained in our work which killed guinea pigs on a dose less than 0.25 c. c. Pigeons were not killed by 0.5 c. c. of filtrate, though 0.1–0.2 c. c. of culture killed these animals in 24 to 48 hours. A good toxin according to Weinberg and Séguin should kill guinea pigs in 1/100 c. c. doses injected intravenously and 1/400 c. c. should kill mice when injected subcutaneously.

It is doubtful whether the culture received is identical with *B. oedematiens* described by Weinberg and Séguin. The cultural characteristics of the organism received do not correspond in all particulars with those described by the above authors. It is stated that the organism is very slightly proteolytic, not digesting blood serum, casein, or ovalbumin. The culture received by us was actively proteolytic comparing favorably in this respect with the Hygienic Laboratory cultures of *B. oedematis maligni*. Casein, blood serum, and minced meat were digested promptly. The failure to produce a potent toxin and the discrepancy as regards cultural behavior indicate that the culture probably was not identical with that used by Weinberg for best toxin production.

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III. POTENCY OF BACTERIAL VACCINES SUSPENDED IN OIL (LIPO-VACCINES).

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During the course of the past year several samples of "lipo-vaccines, that is bacterial vaccines suspended in oil, made from the typhoid and paratyphoid bacilli, also from the pneumococcus, were received at the Hygienic Laboratory for testing. The use of these vaccines has been advocated on the ground that their administration is as effective as that of saline vaccines as a prophylactic measure and that the local and general reactions produced after injection are much milder so that a dose equivalent to or greater than that of the three injections of saline vaccine can be administered at one time.

The following is a preliminary report of some of the work that has been done in an effort to establish standard methods by which tests may be used to determine their efficiency.

TYPHOID-PARATYPHOID OIL VACCINES.

The Hygienic Laboratory method of testing typhoid and typhoid-paratyphoid saline vaccines consists of inoculating rabbits with the usual human doses, giving the three inoculations at intervals of four to five days.[1] In the case of typhoid vaccines the first dose is 500,000,000 and the two succeeding doses 1,000,000,000 organisms. The Hygienic Laboratory typhoid-paratyphoid vaccine contains 2,500,000,000 organisms per mil (c. c.) and the first dose consists of half a c. c. and the other two of one c. c. each. In carrying out the test the rabbits are bled about five days after the last inoculation and the serum tested for agglutinins. Complete or almost complete agglutination should occur in dilutions of about 1/400, and distinct agglutination may often occur in the 1/800 and 1/1,600 dilutions. This applies to *B. typhosus;* in the case of *B. Paratyphosus* α and *B. Paratyphosus* β agglutination occurs in lower dilutions, particularly in the case of *B. Paratyphosus* α.

The typhoid-paratyphoid oil vaccines received for test were inoculated in the usual way into rabbits, the injections being made subcutaneously on the abdomen, three rabbits being used for each test. The human dose 1 c. c. was used, and bleedings were made about ten days after the inoculations.

[1] Hygienic Laboratory Bulletin No. 110.

The following protocols indicate some of the results obtained with the serums of the inoculated rabbits tested for the presence of agglutinins against the three organisms in the vaccines:

TYPHOID-PARATYPHOID OIL VACCINE (from Laboratory 1).

[0.3 mg. *B. Typhosus*, 0.3 mg. *B. Paratyphosus* α, 0.3 mg. *B. Paratyphosus* β per c. c.]

	Final dilutions of serum.																	
	B. typhosus Rawling.						B. paratyphosus α Mears.						B. paratyphosus β Cools.					
	1: 50.	1: 100.	1: 200.	1: 400.	1: 800.	1: 1,600.	1: 50.	1: 100.	1: 200.	1: 400.	1: 800.	1: 1,600.	1: 50.	1: 100.	1: 200.	1: 400.	1: 800.	1: 1,600.
Rabbit 1	1?	1?	1?	0	0	0	0	0	1?	0	0	0	1?	1?	1?	1?	1?	1?
Rabbit 2	1?	1?	1?	1?	1?	1?	0	0	0	0	0	0	1?	1?	1?	1?	1?	1?
Rabbit 3	1	1	1	1	1?	0	0	0	0	0	0	0	1?	1?	1?	1?	1?	1?

HYGIENIC LABORATORY TYPHOID-PARATYPHOID SALINE VACCINE 78.

Rabbit 1	3	3	3	3	2	2	3	3	3	2	1	1?	4	4	4	4	3	3
Rabbit 2	4	4	4	3	1	1?	3	2	2	1	1?	1?	4	4	3	3	3	2
Rabbit 3	4	4	4	3	1	1?	3	3	2	2	1	1	4	4	4	4	3	2
	Control (no serum) 0.						Control 0.						Control 0.					

0.3 mg. *B. Typhosus* signifies 0.3 mg. of dried typhoid organisms per c. c.

The rabbits inoculated with the typhoid-paratyphoid oil vaccine were bled again six days later and showed practically the same results in the agglutination test as those above, i. e., no definite agglutination apparent in any of the tubes.

An oil vaccine made in a second laboratory was tested and the method of injection was varied by inoculating one rabbit subcutaneously in the usual way with 1 c. c., one rabbit with 1 c. c. distributed in four different places, and a third with 2 c. c.

The following protocol shows the results obtained in the agglutination test:

TYPHOID-PARATYPHOID OIL VACCINE (from Laboratory 2.)

[2,500,000,000 each killed *B. paratyphosus* α and β and *B. typhosus* in each c. c.]

	Final dilutions of serum.																	
	B. typhosus Rawling.						B. paratyphosus α Mears.						B. paratyphosus β Cools.					
	1: 50.	1: 100.	1: 200.	1: 400.	1: 800.	1: 1,600.	1: 50.	1: 100.	1: 200.	1: 400.	1: 800.	1: 1,600.	1: 50.	1: 100.	1: 200.	1: 400.	1: 800.	1: 1,600.
Rabbit 1	1?	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Rabbit 2 (4 sites)	1?	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Rabbit 3 (2 c. c.)	1?	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1

HYGIENIC LABORATORY TYPHOID-PARATYPHOID SALINE VACCINE 94.

Rabbit 1	4	3	3	3	3	0	2	2	2	1	1	0	4	4	4	4	4	3
Rabbit 2	3	3	3	1	1?	0	2	2	1	1?	0	0	4	4	4	3	3	2
	Control (no serum) 0.						Control 0.						Control 1					

A test was made by varying the location for injecting the oil vaccine from laboratory 1 with the following results:

1 C. C. SUBCUTANEOUSLY ON ABDOMEN.

	Final dilutions of serums.																	
	B. typhosus Rawling.						B. paratyphosus α Mears.						B. paratyphosus β Cools.					
	1: 50.	1: 100.	1: 200.	1: 400.	1: 800.	1: 1,600.	1: 50.	1: 100.	1: 200.	1: 400.	1: 800.	1: 1,600.	1: 50.	1: 100.	1: 200.	1: 400.	1: 800.	1: 1,600.
Rabbit 1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Rabbit 2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Rabbit 3	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1

1 C. C. SUBCUTANEOUSLY ON THIGH.

Rabbit 1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Rabbit 2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Rabbit 3	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1

1 C. C. INTRAMUSCULARLY ON THIGH.

Rabbit 1	2	1?	0	0	0	0	1?	0	0	0	0	0	4	3	2	2	1	1
Rabbit 2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Rabbit 3	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1

1 C. C. INTRAPERITONEALLY.

Rabbit 1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
Rabbit 2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1

HYGIENIC LABORATORY TYPHOID-PARATYPHOID SALINE VACCINE 97 (3 INJECTIONS SUBCUTANEOUSLY).

Rabbit 1	3	3	1	0	0	0	2	2	2	1	0	0	3	3	2	2	2	1
Rabbit 2	4	4	4	3	1	0	2	2	2	2	1	0	4	4	4	3	2	2
Rabbit 3	4	4	4	3	2	0	2	2	2	1	1	0	4	4	4	3	2	2
	Control (no serum) 0.						Control 0.						Control 1.					

Only one of the rabbits receiving lipo-vaccine in the above test showed any definite agglutination. One of the three rabbits injected intramuscularly showed slight agglutination of *B. typhosus* Rawling, a suggestion of agglutination with the paratyphoid A antigen and very definite agglutination with the paratyphoid B antigen in the lower dilutions.

The above tables indicate that the oil vaccine as administered was not as effective in these tests in producing agglutinins in the animals used as were the saline vaccines. The absorption of these vaccines when injected into the loose subcutaneous tissue of the rabbit probably differs from that in the human subject. The fact that quite definite agglutination was obtained in one instance when the injection was made intramuscularly suggests that this method may be more effective than by the subcutaneous route for producing

agglutinins, but since only one out of three rabbits showed any agglutination, it does not appear that this method can be relied on for uniform results.

PNEUMOCOCCUS OIL VACCINES.

The use of saline pneumococcus vaccine as a prophylactic measure against pneumonia was recently carried out with apparent success on an extensive scale in South Africa. This work has been reported by Lister in the Publications of the South African Institute for Medical Research, 1913–1917.[4]

Following this, prophylactic inoculation against pneumonia by the use of saline vaccines was practiced on 12,519 men of the United States Army during the year 1918, as reported by Cecil and Austin.[5] Three or four doses were administered at intervals of five to seven days, with a total of six to nine billion organisms of types I and II pneumococcus each and four and one-half to six billions of type III. During 10 weeks following the vaccinations no cases of pneumonia caused by these three types occurred among the vaccinated subjects and the incidence against pneumonias caused by type IV was much less than among unvaccinated controls.

Later in the same year pneumococcus oil vaccine was made use of as a prophylactic measure against pneumonia at Camp Wheeler, as reported by Cecil and Vaughan;[6] and 13,460 men, about 80 per cent of the camp strength, received inoculations. In this case 32 cases of pneumonia due to pneumococcus types I, II, and III occurred among the vaccinated 80 per cent, and 42 cases among the unvaccinated 20 per cent. Of the 32 cases of pneumonia due to the types I, II and III all except eight occurred within one week after vaccination and these eight were cases following severe attacks of influenza.

In our tests on the potency of pneumococcus oil vaccine, it was the purpose when the work was begun to inoculate animals or human subjects with the vaccine and after a period thought necessary for the elaboration of agglutinins and protective bodies to test the serum for the presence of these bodies in accordance with the method employed by Cecil and Austin in testing saline vaccines.

Six normal rabbits were bled from the heart and two samples of human blood were also collected. These samples were to be used later as controls in making agglutination and protection tests. Three of the above rabbits were inoculated subcutaneously on the day of bleeding with 1 c. c. each of pneumococcus oil vaccine prepared in Laboratory 1 and three with pneumococcus oil vaccine prepared in Laboratory 3. One of the human subjects was injected with 1 c. c.

[4] Lister, 1913–17. Publications of the South African Inst. for Med. Res. No. II, VII, X.
[5] Cecil and Austin, 1918. Jour. Exper. Med., vol. 28, p. 19.
[6] Cecil and Vaughan, 1919. Jour. Exper. Med., vol. 29, p. 457.

of the first oil vaccine, and the other with 1 c. c. of the second oil vaccine. Agglutination tests of the various sera secured before inoculation were carried out with negative results throughout.

Bleedings of the rabbits and human subjects were made 14 days after inoculation. Agglutination tests were made against 24-hour-old broth cultures of type I, II, and III of pneumococcus, using highly virulent cultures of all three types. No agglutination whatever was obtained in any of the sera tested even with the undiluted serum (dilutions of serum 1:1 to 1:40 were used). A control set of tubes with pneumococcus immune diagnostic serum of each type was run in each case with the following results:

	Dilutions of serum.					
	1:1	1:2	1:5	1:10	1:20	1:40
Type I	4	4	4	4	4	4
Type II	4	4	4	3	3	3
Type III	3	3	3	2	1	0

This showed that the cultures used were readily agglutinable.

Protection tests on mice were carried out with the rabbit sera against broth cultures of the three types of pneumococcus. An equal number of control mice were injected with normal sera of the corresponding rabbits; 0.2 c. c. of serum was injected into each mouse.

The following is a summary of the results obtained:

	Number of mice inoculated.	Number of mice died.
Type I: 0.0001 c.c. to 0.000001 c.c. (test mice)	24	24
Type I: 0.00001 c.c. to 0.0000001 c.c. (control mice with normal serum)	24	24
Type II: 0.00001 c.c. to 0.0000001 c.c. (test mice)	24	13
Type II: 0.00001 c.c. to 0.0000001 c.c. (control mice with normal serum)	24	15
Type III: 0.0001 c.c. to 0.000001 c.c. (test mice)	24	20
Type III: 0.0001 c.c. to 0.000001 c.c. (control mice with normal serum)	24	21

The results in this test show practically no difference in the amount of protection afforded by the sera from vaccinated rabbits and those from unvaccinated rabbits except that in the case of the serum of one rabbit, which had been inoculated with the second oil vaccine, only one mouse out of the six test mice inoculated with type II culture died and four out of the six controls died.

The four surviving rabbits used in the above tests were bled a second time one month after vaccination. Agglutination tests were again entirely negative. Protection tests were made on mice, using cultures of the three different pneumococcus types, all three of which were fatal in doses of 0.0000001 c. c. to 0.00000001 c. c. (in each case both control mice without serum inoculated with 0.0000001 c. c. died,

and one of the two mice inoculated with 0.00000001 c. c. died). The control mice were inoculated with cultures alone. The following summarizes the results obtained:

Type I: All mice died (test mice and control mice).

Type II: The serum from three of the rabbits showed no protection whatever, but in the fourth case all the mice survived, showing there was some protective property in the serum from this rabbit. Somewhat similar results were obtained in the previous test, from this same rabbit when bleedings were made 14 days after inoculations.

Type III: No protection was shown with the serum of any of the rabbits.

Tests were carried out with the sera from the two vaccinated human subjects A (Laboratory 3) and B (Laboratory 1).

SERUM A.

	Number of mice inoculated.	Number of mice died.
Type I: 0.0000001 to 0.00000001 c. c. (mice treated with immune serum)	4	[1] 3
Type I: 0.0000001 to 0.00000001 c. c. (mice treated with normal serum)	4	4
Type I: 0.0000001 to 0.00000001 c. c. (mice receiving no serum)	4	3
Type II: 0.0000001 to 0.00000001 c. c. (mice treated with immune serum)	4	[2] 2
Type II: 0.0000001 to 0.00000001 c. c. (mice treated with normal serum)	4	1
Type II: 0.0000001 to 0.00000001 c. c. (mice receiving no serum)	4	4
Type III: 0.0000001 to 0.00000001 c. c. (mice treated with immune serum	4	0
Type III: 0.0000001 to 0.00000001 c. c. (mice treated with normal serum)	4	3
Type III: 0.0000001 to 0.00000001 c. c. (mice receiving no serum)	4	4

SERUM B.

Type I: 0.0000001 to 0.00000001 c. c. (mice treated with immune serum)	4	3
Type I: 0.0000001 to 0.00000001 c. c. (mice treated with normal serum)	4	4
Type I: 0.0000001 to 0.00000001 c. c. (mice receiving no serum)	4	3
Type II: 0.0000001 to 0.00000001 c. c. (mice treated with immune serum)	4	[1] 1
Type II: 0.0000001 to 0.00000001 c. c. (mice treated with normal serum)	4	0
Type II: 0.0000001 to 0.00000001 c. c. (mice receiving no serum)	4	4
Type III: 0.0000001 to 0.00000001 c. c. (mice treated with immune serum)	4	1
Type III: 0.0000001 to 0.00000001 c. c. (mice treated with normal serum)	4	2
Type III: 0.0000001 to 0.00000001 c. c. (mice receiving no serum)	4	4

[1] Mouse-typhoid infection. [2] 2 Mouse-typhoid infection.

The 12 vaccinated mice treated with human immune serum A showed a probable protection against the types II and III and possible protection against type I. Two of the 12 mice died of undoubted pneumococcus infection and 7 survived (3 mice died of mouse-typhoid infection). Of the 12 vaccinated mice treated with human immune serum B, 4 died of pneumococcus infection (1 of mouse-typhoid infection). A certain amount of protection was apparently afforded by normal serum. Normal serum B, as well as normal serum A, showed rather marked protection against type II, and both showed slight protection against type III. The 12 control mice which received cultures without any serum all died, with one exception (type I, 0.00000001 c. c.).

Tests were carried out by inoculating mice directly with the oil vaccine from laboratory 1, using 1 c.c. of the vaccine injected subcutaneously. Twenty-four vaccinated mice were inoculated with cul-

tures 14 days later. Dilutions of 0.0000001 and 0.00000001 of 24-hour broth cultures of each of these three different types of pneumococcus were used.

	Test mice.		Control mice.	
	Number inoculated.	Number died.	Number inoculated.	Number died.
Type I:				
0.0000001 c. c	4	1	4	4
0.00000001 c. c	4	[1] 1	4	3
Type II:				
0.0000001 c. c	4	[2] 1	4	4
0.00000001 c. c	4	[3] 1	4	2
Type III:				
0.0000001 c. c	4	[4] 3	4	2
0.00000001 c. c	4	[3] 1	4	1

[1] Vaccine not absorbed; mouse-typhoid infection.
[2] Mouse-typhoid infection.
[3] Vaccine not absorbed.
[4] 2 mouse-typhoid infection.

The results show quite definite protection against types I and II.

A further test was carried out on mice with some variations from the methods used above. A number of mice were injected with the pneumococcus oil vaccine from Laboratory 1, in this case using 0.5 c. c. of vaccine instead of 1 c. c. and part of the mice being injected intraperitoneally instead of subcutaneously.

Fourteen days later 36 of the mice vaccinated subcutaneously were inoculated intraperitoneally with 24-hour broth cultures of the three types of pneumococcus, with the following results:

	Test mice.		Control mice.	
	Number injected.	Number died.	Number injected.	Number died.
Type I:				
0.0000001 c. c	6	5	6	6
0.00000001 c. c	6	3	6	6
Type II:				
0.0000001 c. c	6	5	6	6
0.00000001 c. c	6	6	6	6
Type III:				
0.0000001 c. c	6	6	6	6
0.00000001 c. c	6	6	6	6

The test though not as satisfactory as the previous one indicates some protection of the vaccine against type I.

Nine mice vaccinated intraperitoneally were also inoculated with cultures, 0.0000001 c. c. of each type being used throughout. The following indicates the results obtained:

	Test mice.		Control mice.	
	Number injected.	Number died.	Number injected.	Number died.
Type I: 0.0000001 c. c	5	2	6	6
Type II: 0.0000001 c. c	5	4	6	6
Type III: 0.0000001 c. c	5	4	6	6

Rather definite protection against type I is shown in this test and slight protection against types II and III.

Several direct protection tests were carried out on rabbits. In testing the action of pneumococcus vaccine on rabbits it was necessary to determine the virulence of the cultures for this species. Type I was found to be fatal to these animals in the same quantities as to mice, viz, 0.0000001 to 0.00000001 c. c. of culture inoculated intraperitoneally. Rabbits receiving these doses invariably succumbed within 48 hours to a pneumococcus septicemia. Irregular results were obtained with types II and III, indicating that in the case of these two types maintenance of virulence by passage through mice does not necessarily afford a corresponding degree of maintenance of virulence for rabbits. This was particularly true of type III, which sometimes failed to kill in a dilution of 0.01 c. c.

Several rabbits were injected subcutaneously with 1 c. c. of oil vaccine; 14 days later two of the rabbits were injected intraperitoneally with broth cultures of the type I of pneumococcus and two normal rabbits were injected with corresponding amount of culture. The following shows the results obtained:

Test rabbits:
  Type I, 0.00000001 c. c:
    Rabbit 1 survived.
    Rabbit 2 survived.
Control rabbits (culture alone, 0.0000000 1 c. c.):
  Rabbit 3, died in 42 hours.
  Rabbit 4, died in 42 hours.

A test was carried out on mice to determine the relative protection afforded by saline and oil vaccines. Equal numbers of mice were inoculated with saline and oil vaccines, one-half of each group being inoculated with 0.5 c. c. of vaccine and the remaining half with 0.25 c. c. The saline vaccine contained 1,000,000,000 organisms each of types I, II, and III and the oil vaccine 0.7 mg. each of types I, II, and III per c. c.

Eleven days after vaccination the mice were inoculated intraperitoneally with 24-hour broth cultures of the three types of pneumococcus in dilutions of 0.0000001 to 0.00000001. The following summarizes the results obtained. A corresponding number of control mice were inoculated with culture alone at the same time as the vaccinated mice were inoculated with cultures.

OIL VACCINE.

	Number of mice inoculated.	Number of mice survived.	Number of mice died.
Type I: 0.0000001 to 0.00000001 c. c.	8	4	[1] 4
Type II: 0.0000001 to 0.00000001 c. c.	8	2	[2] 6
Type III: 0.0000001 to 0.00000001 c. c.	8	1	[1] 7
	24	7	17

[1] Mouse-typhoid infection.

[2] 2 mixture pneumococcus and mouse-typhoid infection; 1 mouse-typhoid infection.

SALINE VACCINE.

	Number of mice inoculated.	Number of mice survived.	Number of mice dead.
Type I: 0.0000001 to 0.00000001 c. c	8	4	[3] 4
Type II: 0.0000001 to 0.00000001 c. c	9	5	[1] 4
Type III: 0.0000001 to 0.00000001 c. c	7	5	[1] 2
	24	14	10

CONTROL MICE INOCULATED WITH CULTURES ALONE.

Type I: 0.0000001 to 0.00000001 c. c	8	0	8
Type II: 0.0000001 to 0.00000001 c. c	8	0	[1] 8
Type III: 0.0000001 to 0.00000001 c. c	8	1	[1] 7
	24	1	23

[1] 1 mouse-typhoid infection.
[3] 2 mouse-tyhpoid infection; 1 mixture pneumococcus and mouse-typhoid infection; 1 no growth on plate.

Summary.

Oil vaccine:
24 mice inoculated.
7 survived.
12 died (pneumococcus infection).
5 negative or doubtful.

Saline vaccine:
24 mice inoculated.
14 survived.
4 died (pneumococcus infection).
6 negative or doubtful.

The results in this test indicate that the saline vaccine was approximately twice as effective as the oil vaccine. Some protection was definitely afforded by each vaccine, since all the control mice on culture alone died except one (0.00000001 c. c. of type III).

A test was carried out with the pneumococcus oil vaccine from Laboratory 1, Hygienic Laboratory pneumococcus saline vaccine, and a commercial pneumococcus saline vaccine. These contained types I, II, and III of pneumococcus as follows:

Oil vaccine Laboratory 1 0.83 mg. of each type per c. c.
Hygienic Laboratory saline vaccine, 1,000,000,000 each of types I, II, and III per c. c.
Commercial saline vaccine 3,000,000,000 each of types I, II, and III per c. c.

Three series of mice were inoculated subcutaneously with 0.5 c. c. of the respective vaccines; 14 days later the surviving mice received cultures intraperitoneally.

OIL VACCINE, LABORATORY 1.

	Number of mice inoculated.	Number of mice survived.	Number of mice died.
Type I: 0.0000001 c. c	5	1	4
Type I: 0.00000001 c. c	5	3	[1] 2
Type II: 0.0000001 c. c	5	0	[1] 5
Type II: 0.00000001 c. c	5	0	5
Type III: 0.0000001 c. c	5	0	5
Type III: 0.00000001 c. c	5	2	[2] 3
	30	6	24

[1] Doubtful pneumococcus infection.
[2] 1 doubtful, 1 not pneumococcus infection.

HYGIENIC LABORATORY SALINE VACCINE.

	Number of mice inoculated.	Number of mice survived.	Number of mice died.
Type I: 0.0000001 c. c	4	3	1
Type I: 0.00000001 c. c	4	3	[1] 1
Type II: 0.0000001 c. c	4	2	2
Type II: 0.00000001 c. c	4	1	3
Type III: 0.0000001 c. c	4	3	1
Type III: 0.00000001 c. c	4	1	3
	24	13	11

COMMERCIAL SALINE VACCINE.

Type I: 0.0000001 c. c	3	2	1
Type I: 0.00000001 c. c	3	1	2
Type II: 0.0000001 c. c	3	3	0
Type II: 0.00000001 c. c	3	2	1
Type III: 0.0000001 c. c	3	0	3
Type III: 0.00000001 c. c	3	2	1
	18	10	8

CONTROL MICE INOCULATED WITH CULTURES ALONE.

Type I: 0.0000001 c. c	5	0	5
Type I: 0.00000001 c. c	5	0	5
Type II: 0.000000 c. c	5	0	5
Type II: 0.00000001 c. c	5	0	5
Type III: 0.0000001 c. c	5	0	5
Type III: 0.00000001 c. c	5	3	2
	30	3	27

[1] Doubtful pneumococcus infection.

Protection was afforded against the cultures for slightly more than half of the mice treated with saline vaccine, and for less than one-third of the mice inoculated with the oil vaccine.

CONCLUSIONS.

Tests have been carried out with certain oil vaccines for the purpose of establishing methods of standardizing the potency testing of these products. The preliminary work is here reported, but as yet not sufficient data have been obtained to justify the establishment of any definite standard or method of testing.

In the case of the typhoid-paratyphoid oil vaccines the adaptation of the Hygienic Laboratory method of testing saline vaccines on rabbits for the production of agglutinins did not give results which compared favorably with those of saline vaccines, as far as carried out.

Pneumococcus oil and saline vaccines were tested on mice and rabbits. A few tests with the oil vaccine on human subjects were also made. The results obtained in the case of rabbits and mice indicate that though both afford a certain amount of protection, the saline vaccine was rather more effective in these animals. Protection tests made with immune sera from human subjects showed somewhat more favorable results than corresponding tests carried out with immune sera from rabbits, though testing on human subjects is not always a practical method for testing products.

The results of these tests which were performed solely as a study in standardization should not be interpreted as having any necessary bearing on the prophylactic use of oil vaccines to prevent infection in man.

HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

Of the bulletins published by the laboratory since its establishment, copies of the following are available for distribution and may be obtained without cost by applying to the Surgeon General, United States Public Health Service, Washington, D. C.:

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin (an American unit established under authority of the act of July 1, 1902). By M. J. Rosenau and John F. Anderson.

No. 46.—*Hepatozoon perniciosum* (n. g., n. sp.); a hæmogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*Ielaps echidninus*). By W. W. Miller.

No. 50.—Further studies upon the phenomenon of anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 51.—Chemical tests for blood. By Joseph H. Kastle.

No. 52.—Report No. 3 on the origin and prevalence of typhoid fever in the District of Columbia (1908). By M. J. Rosenau, Leslie L. Lumsden, and Joseph H. Kastle.

No. 55.—Quantitative pharmacological studies; adrenalin and adrenalin-like bodies. By W. H. Schultz.

No. 59.—The oxidases and other oxygen catalysts concerned in biological oxidations. By Joseph Hoehing Kastle.

No. 65.—Facts and problems of rabies. By A. M. Stimson.

No. 73.—The effect of a number of derivatives of choline and analogous compounds on the blood pressure. By Reid Hunt and R. de M. Taveau.

No. 75.—Digest of comments on the Pharmacopœia of the United States of America (eighth decennial revision) and the National Formulary (third edition) for the calendar year ending December 31, 1908. By Murray Galt Motter and Martin I. Wilbert.

No. 76.—The physiological standardization of ergot. By Charles Wallis Edmunds and Worth Hale.

No. 78.—Report No. 4 on the origin and prevalence of typhoid fever in the District of Columbia (1909). By L. L. Lumsden and John F. Anderson. (Including articles contributed by Thomas B. McClintic and Wade H. Frost.)

No. 81.—Tissue proliferation in plasma medium. By John Sundwall.

No. 86.—Studies on typhus. By John F. Anderson and Joseph Goldberger.

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No. 89.—Sewage pollution of interstate and international waters with special reference to the spread of typhoid fever. VI. The Missouri River from Sioux City to its mouth. By Allan J. McLaughlin.

No. 91.—I. The cause of death from subdural injections of antimeningitis serum. By Worth Hale. II. Some new cholera selective media. By Joseph Goldberger.

No. 94.—I. Collected studies on the insect transmission of *Trypanosoma evansi*. By M. Bruin Mitzmain. II. Summary of experiments in the transmission of anthrax by biting flies. By M. Bruin Mitzmain.

No. 95.—Laboratory studies on tetanus. By Edward Francis.

No. 96.—1. Report of investigation of coastal waters in the vicinity of Gulfport and Biloxi, Miss., with special reference to the pollution of shellfish. By R. H. Creel. 2. A comparison of methods for the determination of oxygen in waters in presence of nitrite. By Elias Elvove. 3. Some new compounds of the choline type. III. Including preparation of monoacetate of *a*, *B* dioxy-*B*-methyl butane. By G. A. Menge. 4. The detection of white phosphorus in matches. By Earle B. Phelps. 5. The chemical composition of rubber in nursing nipples and in some rubber toys. By Earle B. Phelps and Albert F. Stevenson. 6. The analysis of thymol capsules. By Atherton Seidell. 7. Seasonal variation in the composition of the thyroid gland. By Atherton Seidell and Frederic Fenger. 8. Note on a new apparatus for use with the Winkler method for dissolved oxygen in water. By Hyman L. Shoub. 9. The pharmacological action of some serum preservatives. By Carl Voegtlin.

No. 97.—1. Some further siphonaptera. 2. A further report on the identification of some siphonaptera from the Philippine Islands. 3. The taxonomic value of the copulatory organs of the females in the order of siphonaptera. By Carroll Fox.

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No. 119.—Digest of comments on the Pharmacopœia of the United States of America and on the National Formulary for the calendar year ending December 31, 1916. By A. G. Du Mez.

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