THE BACTERIOLOGICAL DIAGNOSIS OF CHOLERA.

A REPORT PRESENTED TO THE PERMANENT COMMITTEE OF THE INTERNATIONAL OFFICE OF PUBLIC HYGIENE IN THE NAME OF A COMMISSION COMPOSED OF: MESSRS. RUFFER, president; CALMETTE, GAFFKY, GEDDINGS, MURILLO, PRAUM, AND POTTEVIN, reporter.

Translated from the French by H. D. Geddings, Surgeon, Public Health and Marine-Hospital Service, and American delegate on the permanent committee of the International Office of Public Hygiene.[1]

CONCLUSIONS.

The methods applicable at the present time to the investigation and identification of the cholera vibrio permit the skilled bacteriologist to positively assert a diagnosis of cholera under conditions of sufficient certainty for the needs of prophylaxis.

Practically the rule may be adopted to consider as truly choleraic every choleriform vibrio in which one or the other of the two following characteristics is recognized:

1. Agglutination in the proportion of 1 to 1,000 at least, by a cholera serum, the activity of which is 1 to 4,000 or more.

2. Positive Pfeiffer reaction.

Every choleriform affection presenting symptoms of gastro-enteritis in which is encountered a vibrio answering to this definition, should hence be considered as a case of cholera.

When it is a question of first suspicious cases appearing in a country heretofore free of cholera, it is indicated to prove the vibrios by one or the other of the two reactions above given.

If in a locality there appear repeated attacks of a disease presenting the clinical symptoms and anatomo-pathologic lesions of cholera and the bacteriological examination reveals in the feces of the patients and in the intestinal contents of cadavers a choleriform, vibrionic flora, even if the vibrios isolated are not agglutinable in the proportion of 1 to 1,000 and do not give the Pfeiffer reaction, it is indicated in these exceptional conditions to repeat the bacteriological examinations until all doubt is removed.

The proof of the existence of the cholera vibrio in persons presenting no clinical signs or anatomo-pathologic lesions does not necessitate a declaration of the existence of cholera.

---

[1] The International Office of Public Health was established as the result of an agreement between the countries signatory to the arrangement signed at Rome, Dec. 9, 1907. The work of the office was placed under the authority and control of an international committee composed of technical representatives designated by the participating countries, one representative for each country. The committee assembled for the first time at Paris, Nov. 4, 1908. The members of the committee keep in touch with the office at all times, in order that they may be able to decide intelligently the questions which may from time to time arise.

Bacteriology has not at its disposal at the present time any reactions permitting the establishment in a definite, practical manner of the differentiations between various cholera vibrios.

It would be neither possible nor desirable to fix a technique limited by strict rules for the various operations of bacteriological examinations, but the following general indications may be recommended as permitting in the great majority of cases a positive diagnosis within 24 to 36 hours:

1. When mucous flakes are available for examination a microscopical investigation of the same, in stained preparations and in the hanging drop.

2. The isolation of the vibrios, employing for the purpose agar media, at a temperature of 37° C.

(*a*) Plant plates of ordinary suitably alkalinized agar and of Dieudonné's medium, using for the latter a risiform particle, or an equivalent quantity of feces.

(*b*) Plant in 50 c. c. of peptone solution 1 c. c. of fecal matter. After a stay of six hours in the incubator (or 12 to 18 hours if need be), at 37° C. take several loopsful from the surface and plant with them several plates of Dieudonné medium and ordinary agar.

(*c*) Investigate the agglutination reaction, using drops for the purpose, from the isolated colonies, the properties belonging to cholera vibrios, and secure pure cultures.

3. Demonstrate the character of the vibrios obtained in pure culture, by the reaction of agglutination or that of Pfeiffer.

The conditions are much more favorable to the discovery of vibrios if pathological materials (feces or intestinal contents) are collected as early in the attack as possible, or secured from the cadaver as early as possible after death. Examinations made of the small quantity of material collected by a sound introduced into the rectum, in the living body, or from the cadaver are unreliable. It is sometimes possible to recognize that a person even in good health has undergone an attack of cholera by determining whether his blood serum gives with a genuine cholera vibrio the immunity reactions, viz, agglutination or the reaction of Pfeiffer.

(*The above conclusions were unanimously adopted by the commission. At the meeting of October 9, 1911, the permanent committee likewise adopted them unanimously.*)

DISCUSSION AND HISTORICAL REVIEW.

The bacteriological diagnosis of cholera is established by the finding of the specific vibrio, discovered by R. Koch, in the suspected products (feces, intestinal contents, sometimes vomited matter). To be justified in pronouncing a positive diagnosis, the vibrio must have been isolated, obtained in pure culture, and recognized by its reactions.

We may recognize an attack of cholera by those reactions which the blood of the patient gives with the specific vibrio. But the property of giving these reactions, besides being inconstant, only appears slowly, while on the contrary it persists for quite a long time after cure, and often permits the establishment of a retrospective diagnosis.

I.

When cholera shows itself with its classical gastro-intestinal syndrome, the rice-water stools of the acute period represent very frequently cultures pure, or almost so, of the vibrio of Koch. It is specially abundant in the desquamated masses of cells which constitute the so-called "grains of rice."

If the disease is prolonged, in proportion as the acute stage is left behind, the ordinary bacteria of the intestine appear, and predominate more and more in the feces, as well as in the intestinal contents. In benign or atypical cases, among bacillus carriers, healthy or convalescent from cholera, the vibrios may be very scarce, and represent sometimes a few individual organisms, nested in the mass of bacteria which constitute the intestinal flora. Their discovery is then very delicate, and demands all the competence of the skilled bacteriologist.

At the necropsy of subjects dead during the acute period the vibrio is met with in abundance in the contents of the small intestine, principally in the layer of flaky mucus, formed of desquamated epithelial cells, which coats the surface of the intestine. It may penetrate into the intestinal walls and invade the cells of the villi and tubular glands, or even reach the superficial layers of the lymphatic follicles. It is not to be found in the other organs or in the blood.

According to observations recently made in man, and in cases of experimental cholera in the young rabbit, the vibrio may invade the biliary passages. Its persistence in these organs may be the origin of the prolonged excretions which are sometimes noted among bacillus carriers.

Microscopical examination of pathological products may often furnish in a few moments very useful indications (1):[1]

Diluting a little of the product to be examined in a drop of sterile water or bouillon and examining between slide and cover glass, preferably with ultra-microscopic illumination, the vibrios may be discerned and recognized by the peculiar liveliness of their movements. Colored preparations, made in the beginning from flakes of mucus, often preserve a characteristic appearance, to the importance of which Koch drew particular attention:

According to the gravity of the case and the stage of the disease, the choleraic bacteria are met in preparations of this sort, perhaps in absolutely pure or nearly pure culture, perhaps mixed with ordinary intestinal bacteria, principally the *Bacterium coli*, in all possible proportions up to those in which, microscopically, the presence of curved rods can no longer be revealed.

If one has to do with a pure culture of cholera bacilli, or if the latter are accompanied only by the *Bacterium coli* but are themselves in superior number, then the cholera bacilli are as a general rule grouped in the places where they have been deposited in filaments from the mucus, in groups of a characteristic form. In fact they constantly constitute little masses, in which all the bacilli have uniformly the same direction, so that one might believe that he was looking at a troop of these bacilli following each other in files, like fish in still water. If in preparations the characteristic groupings are wanting, but if, however, the vibrios appear in culture, pure or nearly so, it may be considered as extremely probable that one is dealing with a case of cholera (2).

In 1905 the Institute for Infectious Diseases in Berlin examined 2,565 samples of suspected pathological products, with 251 positive results. In 33 cases (13 loops of intestine and 20 specimens of stools)

---

[1] For explanatory notes to which the numbers in parenthesis refer, see end of report.

the microscopic examination alone led to a positive diagnosis. This was confirmed 30 times by subsequent investigations. Twice the vibrios isolated showed themselves nonagglutinable, and once it was impossible to obtain a culture of the vibrios which had been shown in the preparation.

It is only in those cases where the vibrios are very abundant in the products submitted to examination, that direct planting upon solid media renders it possible to obtain at the first stroke pure and isolated colonies. As a general rule it is necessary to have recourse to preliminary cultures of enrichment, and these ought to be put in course of preparation, systematically and at the beginning of each research (3).

It is sufficient generally to plant in peptone solution about 1 cubic centimeter of fecal matter. But as it may happen that a trial made under these conditions remains negative, vibrios may still be isolated when a larger quantity is used; 5 cubic centimeters, for example.

In some cases, however, a positive result can not be obtained except by making the experiment upon much larger quantities. Gaffky relates that in six researches made upon materials collected at necropsy examinations made according to the ordinary methods having remained negative, in four instances he succeeded in isolating the cholera vibrio by planting the entire contents of a loop of intestine in a liter of peptone water.

For a long time gelatine media were the most used for the isolation of cholera vibrios, starting from the original material or from enrichment cultures in peptone water. Hence, specially for the reason of the typical appearance which in general surrounds colonies of cholera vibrios in such media, they have been considered as constituting an important diagnostic element (4).

Gelatin plates had also another interest. R. Koch had defined cholera as "a state peculiar to man in which the intestine contains vibrios, cultivable in 10 per cent nutrient gelatin, and producing therein a moderate liquefaction," and it is certain that no other human disease is known, producing feces the planting of which upon gelatin plates gives rise to such a development of liquefying colonies. The acute gastro-enterites due to paratyphoid infections, or to food poisonings, which are the most frequent among those simulating the choleraic syndrome, subjected to bacteriological test, deport themselves in an entirely different fashion.

To-day the elements of diagnosis, drawn from the more or less characteristic appearance of plates or colonies, have only a quite secondary value compared with the precise and certain indications furnished by immunity reactions. Gelatin media have no other interest, therefore, than permitting a practical separation of vibrionic colonies. From this point of view they are inferior to agar-agar media, with which operations made at 37° C. furnish equally good results in a much shorter time.[1]

---

[1] For all operations relating to the bacteriologic diagnosis of cholera it is indicated to use an agar-agar of an alkaline reaction. A very convenient degree of alkalinity is obtained by adding to each 100 c. c. of medium, neutral to litmus, 3 c. c. of a 10 per cent solution of sodium carbonate.

The colonies of the cholera vibrio upon ordinary agar-agar are less characteristic than those upon gelatin; nevertheless a practiced eye can often recognize them in the midst of colonies belonging to the common bacteria of feces. They form upon the surface of the agar flat disks, transparent, and of a grayish blue color, while the others are globular and opaque. They can be made use of at the end of six to eight hours in the incubator at 37° C. The plantings ought to be made in streaks, or by flooding the surface of the solidified medium.

In order to render the research of vibrionic colonies upon plates easier, by eliminating as far as possible the common species, various elective media have been proposed.

In a series of publications the first of which goes back to 1893, Deycke had attempted to have adopted a medium elective for cholera, the principle of which was the employment of an alkaline solution of gelatin, with the addition of 0.75 per cent of soda; but its use never became general. In 1909 Dieudonné published the formula of a new medium, which had more success, and which has been found to have added a real perfection to the methods employed in the search for vibrios (5).

The medium of Dieudonné has been largely used in the course of cholera epidemics in 1910, notably in Italy. This is the opinion expressed by Gosio in a communication to the Academy of Medicine of Rome upon the value and the conditions of its use:

The total number of researches made upon feces coming from cholera patients, from suspects, from convalescents, and from persons simply exposed to the contagion, amounts to several thousands. The results have shown in an incontestable manner the advantages of the method.

From the point of view of prognosis of cases, it has been possible to distinguish, according to whether the vibrios are few or numerous.

When the vibrios are abundant, as is the case with cholera feces, colonies planted directly upon the blood agar, with one loopful per plate, were already visible to the naked eye and could be used for specimen plantings at the end of 6 to 8 hours. In favorable cases, in less than 12 hours, the vibrio of cholera could be isolated and identified by agglutination.

There exist microorganisms which develop upon the Dieudonné medium as well as the cholera vibrios. Such were certain specimens of colon, certain cocci and coccobacilli, and at Barletta, toward the end of the epidemic, a small diplobacillus became exceedingly common on the plates.

When the vibrios are few in number, as happens among convalescents and bacillus carriers, direct planting (feces to plates) is not to be recommended, as giving in every case less constant results. It is better in these cases to make a preliminary enrichment culture in peptone water, and to practice subsequently the separation upon the blood agar, which thus preserves all of its advantages.

Crendiropoulo and Madame Panayotatou endeavored to find a medium which would permit the elimination of the *B. pyocyaneus*, which in the course of the cholera epidemic of 1902 had shown itself particularly annoying in the researches for vibrios, to which it added sometimes insurmountable difficulties. They succeeded in making one upon which the pyocyaneus had scarcely commenced to grow at the end of 36 hours, while the vibrios gave useful colonies in 18 hours (6).

Up to the last few years, apart from the characteristics already mentioned as to morphology and appearance of cultures on gelatine and agar, the production of indol and the action of cultures upon laboratory animals were considered as indispensable to a diagnosis (7).

It used to be held that the property of giving the indol reaction belonged to all species of cholera vibrios. This rule may be regarded to-day as very general, though not absolute. There are exceptions. Thus, for example, the observations collected at St. Petersburg in 1908–9 show that of 588 specimens of specific vibrios of human origin, and proved as such by the immunity reactions, 55, or very nearly 10 per cent, did not give the reaction. Among the vibrios originating in waters, the proportion of nonreacting rose to 20 per cent.

It was also considered as an important character of the cholera vibrio to possess a strong virulence for the guinea pig, by intraperitoneal injection. It is known to-day that some vibrios, which by their origin as well as by their immunity reactions are to be regarded as those of true cholera, are almost completely inactive, while vibrios, positively noncholeraic, on the contrary, show themselves extremely virulent.

A number of vibrios are pathogenic for pigeons on deep intramuscular injection, while this property is generally wanting in vibrios isolated from choleraic stools. This is a negative characteristic which merits to be held in mind.

Experimentation by the mouth upon the spermophile or the young rabbit, which are susceptible to contracting an infection very similar to human cholera, is not a procedure generally practicable in current research. Moreover, the indications furnished have not as precise a significance as might a priori be assumed. The fact that a microbial culture may produce a choleriform infection in the spermophile or the young rabbit is not to be interpreted per se as meaning that it can give rise to an epidemic of human cholera. To justify such a conclusion there should be an aggregation of facts, epidemiologic and experimental, to show that such infections can not result from the absorption of common organisms. Now, these facts do not at present exist in science. The results obtained by Metchnikoff with the vibrios of Versailles, of which later mention will be made, are in favor of a contrary opinion.

From all that has been previously said it may be concluded that morphological characteristics, cultural peculiarities, and facts drawn from the action upon laboratory animals, do not furnish the means of certainly defining the cholera vibrio. None of them in fact is fixed and pathognomonic. Moreover, there have been isolated from many media, notably waters, very many vibrionic species which can not be distinguished from those isolated from cholera intestines, and which yet can not in view of their frequency of occurrence be considered as on any account capable of producing cholera.

These uncertainties of diagnosis which have been revealed by science, and the specificity of the Koch vibrio and epidemiologic facts, such as the existence of healthy bacillus carriers, the most modern researches only have been able to confirm. All this simply means that the chances of error of which it is necessary to take account, are not as great as might be believed, and are no greater than can be in a large measure corrected by the skill of the bacteriologist and his highly specialized competence.

The question of the identification of the cholera vibrio has entered upon a new phase with the introduction into bacteriological technique of the immunity reactions, as a consequence of the labors of Pfeiffer and Isaief, of Gruber and Durham, and of Bordet.

The serum of an animal immunized against a vibrio exercises upon this vibrio a specific action, which is manifested when it is added to a vibrionic emulsion, by the immobilization of the microbes; their agglutination into large flakes, leaving a clear liquid; and finally by the disintegration (bacteriolysis) of their cells, which are partially dissolved, and of which only spherical granules remain.

The specific activity of a serum may be quantitatively expressed by the extent of the dilution in which it is capable of producing the

phenomena of agglutination and bacteriolysis, under given conditions and in a given time.

The first experiments made, notably by Dunbar, in order to determine whether the manner in which it acted in the presence of the serum of an animal immunized with a cholera vibrio would give a diagnostic point useful in the identification of a suspected vibrio, had already given positive results. But it was the fundamental work published in 1903 by Kolle and Gotschlich, with the collaboration of Hetsch, Lentz, and Otto, which definitely established the high practical value of the bacteriolysis (reaction of Pfeiffer) and agglutination, for the bacteriological diagnosis of cholera (8).

The work of Kolle and E. Gotschlich was carried out on 87 species of vibrios; 77 had been isolated by Gotschlich at Alexandria during the epidemic of 1902, from patients or suspects; 4 were well known species of Koch vibrios, which by reason of their origin and their characteristics must be considered as truly choleraic; 1 in particular (*vib. Pfeiffer*) had produced an accidental choleraic infection, absolutely typical and very severe; 2 were paracholeraic, well known (*vib. Metchnikkovi* and *vib. Nordhafen*).

Agglutination experiments with the serum of rabbits, normal or immunized with the vibrio of Pfeiffer gave the following results:

Normal serum only produced agglutination in very large doses, which were practically the same for all vibrios.

Immune serum permitted the classification of vibrios into two distinct groups, comprising:

GROUP A.—Vibrios which were only agglutinated by doses of the serum such as it was necessary to employ when operating with the normal serum (1–20 to 1–50). None of them gave the reaction of Pfeiffer. Some of them presented the general characters of the vibrio of Koch; others differed from it clearly. Several were polyciliated.

GROUP B.—Vibrios agglutinable by very weak doses of serum (1–1,000 to 1–20,000). All the vibrios of this group, which comprised four species considered as choleraic, presented the characters of the vibrio of Koch, and were monociliated. All of these for which the Pfeiffer reaction was tried reacted positively.

Agglutination experiments, controlled, made by trying against all vibrios, the serum of rabbits vaccinated with one of them, led to the conclusion that when the immunizing vibrio was one of group A, the agglutinating power was evident with that microbe alone, or exceptionally with one or two others. For the serums prepared with vibrios of group B the agglutinating power was not sensibly displayed against those of group A, but in group B there was no difference to be noted between the immunizing vibrio and the others; the power was manifested against all in the same dilutions.

These facts are clearly shown in the tables below given:

TABLE I.—*Maximum agglutinating power for the normal serum of various species of animals against the vibrios of groups A and B, the immunization being made in all cases with the v. Pfeiffer.*

	Goat.		Ass.		Horse.		Rabbit.		Ox.	
	N. S.	I. S.	N. S.	I. S.	N. S.	I. S.	N. S.	I. S.	N. S.	I. S.
Group A............	200	200	50	100	200	200	50	50	50	(?)
Group B............	50	2,000	20	5,000	200	2,000	20	20,000	50	(?)

N. S.=Normal serum.   I. S.=Immune serum.

TABLE II.—*Agglutinative power of immune serums from rabbits immunized with one of the vibrios of group B in the presence of various vibrios of the same group.*

Number of vibrios used in immunization of animals.	Numbers of vibrios subjected to agglutination test.										
	I	II	VI	VII	XI	XII	XIX	XXII	XXVI	XXVII	XXIX
I..........	5,000	20,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	2,000
II..........	5,000	20,000	1,000	1,000	1,000	1,000	1,000	1,000	2,000	1,000	1,000
VI..........	10,000	20,000	2,000	2,000	5,000	2,000	5,000	2,000	5,000	5,000	2,000
VII..........	10,000	20,000	5,000	2,000	2,000	1,000	1,000	1,000	2,000	5,000	5,000
XI..........	5,000	20,000	5,000	2,000	2,000	2,000	5,000	10,000	5,000	5,000	5,000
XII..........	20,000	20,000	10,000	10,000	5,000	2,000	5,000	2,000	20,000	20,000	20,000
XIX..........	2,000	10,000	1,000	1,000	2,000	2,000	1,000	2,000	2,000	1,000	1,000
XXII......	5,000	20,000	5,000	2,000	5,000	2,000	2,000	2,000	2,000	2,000	2,000
XXVI......	5,000	20,000	5,000	1,000	2,000	1,000	2,000	1,000	2,000	2,000	2,000
XXVII.....	10,000	20,000	1,000	1,000	2,000	2,000	2,000	1,000	2,000	2,000	2,000
XXIX.....	5,000	10,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	5,000

One would therefore be constrained to think that the organisms of group B constitute one and the same species, viz, the cholera vibrio the others representing distinct species without any relation to it.

This conception has been taken as a base for the bacteriologic diagnosis of cholera, not only by laboratories of scientific research, but also in the regulations established by sanitary administrations. Innumerable observations collected in the past eight years have established in the most absolute manner its high practical value.

It has therefore been established on the one hand that the indications furnished by the two reactions of agglutination and bacterioiysis are always in perfect concordance; on the other hand, that every one of the vibrios which react positively in the presence of a cholera serum, when used for the immunization of animals, gives a serum active for all the others, and for these alone. These facts which apply to thousands of vibrios isolated from positive cases of cholera, under the most varied conditions of time and place, are thus very much in favor of a characteristic, specific unity to be attributed to the entire group of vibrios which give the positive reaction.

In the case of characteristically clinical cholera, happening in the midst of epidemics, vibrios of positive reaction are, so to speak, never wanting. Where in the case of repeated analyses, carried out on pathological material of the acute period, they have not been found it is to be considered that their number does not exceed the total of failures

which must be counted on, with even the very surest methods of bacteriological research.

Among healthy individuals vibrios with positive reactions are encountered, especially in the vicinity of cholera patients. Outside of such conditions they are found more rarely in proportion as the researches are made upon individuals among whom the chances of specific infection are more uncertain.[1]

As to media outside of the human body, vibrios of positive reaction have hardly ever been found save under conditions where soiling by choleraic products was, if not evident, at least possible.

Although everybody is in accord in recognizing the diagnostic value of the immunity reactions, especially that of agglutination, which by reason of its ease of execution is generally employed, this standard has not been everywhere adopted as regards the interpretation of the results of experiments.

The German regulations of 1907 specify that the agglutination should be considered as positive and should be regarded as showing the culture to be truly choleraic when a clear agglutination is produced in dilutions corresponding to the limit of power of the immune serum employed.

The instructions of the Italian Government consider as choleraic every vibrio which is proved to be agglutinable in a dilution corresponding to the maximum power of the serum.

In France the instructions addressed to the laboratories charged with the examination of suspected material in 1910 directed that a vibrio should be considered as choleraic which was agglutinated by a serum in a dilution of 1–2,000, the power of which was in the proportion of 1–4,000, or thereabouts.

It would be profitable to eliminate the differences, and to settle upon a basis of interpretation which would be acceptable to all.

Any rule which has for its end to regard as conclusive only those agglutinations produced in dilutions corresponding to the extreme power of the serums appears to us to be too delicate.

Table II shows that in the experiments of Kolle and Gotschlich the vibrios of group B presented very variable agglutinabilities in the presence of the same immune serum, and that the most agglutinable were not necessarily the homologous vibrio.

The order in which they might be classified in the order of their increasing sensitiveness does not always remain the same when the serum used in the experiment varies.

---

[1] The following figures are given for the sake of example:

(a) At St. Petersburg, from Dec. 4, 1908, to Dec. 4, 1909, there was examined the fecal matter of 9,357 individuals isolated on account of the appearance of cholera in the apartment in which they dwelt; 577 were found to be carriers of the specific vibrio. From Dec. 4, 1909, to Dec. 2, 1910, out of 3,173 persons examined, and who had been exposed to the infection in proximity to choleraic patients, there were found:

	Number of persons examined.	Total number of bacillus carriers.	Percentage of carriers.
Adults	2,368	157	6.6
Children, 1 to 15 years	720	71	9.8
Children under 1 year	85	17	20.0

(b) The systematic examinations practiced in Germany in 1910 at the frontier upon all boatmen coming from Russia revealed only 3 carriers of bacilli among more than 5,000 individuals.

At Naples in 1910 examinations were made of the feces of all emigrants embarking for America who came from infected communes. Out of about 2,000 individuals, 12 carriers of specific vibrios were discovered.

Under these conditions it is obvious that the determination of the extreme power of a serum is found to be dependent upon the choice which may be made of the vibrio chosen for the experiment, and a source of error may result in the diagnoses to be subsequently made with this serum.

If serum XII is taken as an example: In testing it with the cultures I, II, XXVI, XXIX, and applying at the end with rigor the rule of the extreme of power, it would be found that it would lead to considering as noncholeraic a number of vibrios given in the table, and especially the very vibrio which was used in the preparation of the serum.

Moreover it must be borne in mind that the interpretation of an agglutination comprises, as was shown by Ruffer at the last session of the international committee, a personal coefficient which is not to be neglected. The extreme limit of the same serum, under conditions otherwise identical, may vary with different bacteriologists.

The results of the experiments of Kolle and Gotschlich, summarized in Tables I and II, as well as innumerable other observations made in the course of the last few years, warrant the deduction of the following conclusions: With serums strongly agglutinative, the power of which reaches 1–4,000, the agglutinative power for common vibrios does not as a general rule exceed 1–50, rarely reaching 1–200; agglutinations in dilutions of 1–500 have been only very exceptionally observed. Cholera vibrios, on the contrary, are found to be agglutinable in dilutions varying between 1–1,000 and 1–20,000.

It appears, therefore, that a rule might with advantage be adopted to regard as choleraic every vibrio which is agglutinated in 1–1,000 at least by a serum the activity of which is 1–4,000 or over. For vibrios agglutinable by a cholera serum only in stronger dilutions comprised, say, between 1–1,000 and 1–500, the results should be considered as doubtful.

As concerns the Pfeiffer reaction, consideration should be had for the indications given previously in the note on technique, when the vibrio permits recourse to the procedure of Pfeiffer; in other cases the reaction should be made according to the method of Bordet, using the same basis for the interpretation of results.

II.

The practical value of determination of species, based upon the immunity reactions being recognized and regarded as beyond discussion, can it be considered that its adoption resolves in a complete manner the problem of the identification of the cholera vibrio?

The question may be considered as leading to two others, which may be thus expressed:

1. If in the analysis of suspected materials there are found vibrios in which the reaction of agglutination and the Pfeiffer reaction are negative, even though they be in pure culture or at least in sufficient abundance to justify the suspicion of a vibrionic infection, ought the diagnosis of cholera nevertheless to be excluded on the ground that they are positively incapable of producing a choleraic infection?

2. Ought all vibrios of positive reaction to be considered as choleraic in the same force in spite of the fact that bacteriology can not establish differentiations between them, based upon other characteristics, to which in practice it is necessary to pay some attention?

These questions must now be considered successively.

A number of observations which have been made in relation to epidemics, and in scientific literature, tend to show that when one is confronted by a suspected case in which bacteriological examination reveals the presence of a vibrionic flora, and the vibrios found are not agglutinable, it would be imprudent to commit one's self to a negative diagnosis without the most ample information.

The old authors were all in accord in recognizing that in the course of a cholera epidemic diarrheas were very frequently observed in which it was impossible to assert whether they were of a specific or ordinary nature. Often after one or two days they evolved into an attack of true cholera, and were then spoken of as preliminary diarrhea.

As Gaffky remarked at the last session of the committee, these diarrheas of doubtful diagnosis, which were formerly very numerous, notably at Hamburg in 1892, are now much more rare, thanks to the perfection of bacteriological technique, but they have not as yet totally disappeared from our statistics.

In 1910 the city of St. Petersburg was free from cholera for the month of January. But between January and June, the time at which the first new cases of cholera were demonstrated and bacteriologically confirmed, cases of gastro-enteritis were received in hospital, some acute, and sometimes followed by death. In April these cases went on increasing in very large proportion, but bacteriological examination did not always show any vibrios, or, if they were found, they were not agglutinable by a specific serum. Later, when cholera had been declared epidemic, the cases of gastro-enteritis without specific vibrios did not cease. The two epidemic manifestations (as had already been the case in 1908 and 1909) continued side by side.

The total number of cases of acute gastro-enteritis observed in 1910 was 1,849, as compared with 4,600 cases of cholera. It sometimes happened that in the same house there were cases of cholera and cases classed as gastro-enteritis.

It could not but be thought that a number of the cases of gastro-enteritis observed before, during, and after the epidemic were themselves also of choleraic nature.

Several times researches made in St. Petersburg, in the contacts of cholera patients or among persons coming from infected districts, showed the presence of carriers of nonagglutinable vibrios in numbers equal to or even higher than carriers of the agglutinable vibrios.

In 1908, at Manila, McLaughlin found out of 376 persons exposed to cholera infection 27 carriers of specific vibrios and 46 carriers of the nonspecific varieties.

In 1911, at Alexandria, the feces of 5,050 persons coming from infected countries were examined. Twelve carriers of specific vibrios and 14 carriers of the nonspecific were found.

It has already been stated that the work of Kolle and Gotschlich was carried out upon 77 vibrios isolated during the epidemic of 1902 in Egypt from the stools of persons presenting suspicious symptoms of cholera or having been in contact with cholera patients; 18 of these vibrios did not enter into the group of true cholera. They came from 14 persons whom, in their memoirs, Gotschlich classes as follows: Five were certainly choleraic; five were apparently not. As to the other

four, nothing could be said in view of the insufficiency of the information.

Thus, therefore, in cases of certain cholera bacteriological examination would have disclosed only nonspecific vibrios. According to Kolle and Gotschlich, this would be due to errors of manipulation. Isolations upon plates would have given in special cases mixtures of vibrionic colonies, belonging some of them to specific species and others to common varieties, and it would have been found that at the moment of transference some plantings would have been taken accidentally from common varieties. This hypothesis admitted, it would be interesting to verify by experiments made by examining each time a sufficient number in order that in cases where nonagglutinable vibrios were found it might be decided whether they existed accidentally alongside of specific vibrios or whether they constituted, themselves alone, the entire vibrionic flora; and, as a second alternative, in seeking by means of reciprocal reactions of these vibrios and the serum of patients whether they might not be considered as the true agents of the infection.

Certain peculiarities would appear to indicate that such researches would present a special interest, had they been followed, in an edemic country, during the periods which precede the beginning of an epidemic of cholera, and upon all first cases.

It is a very general rule that the biologic properties of a microbial species vary in a large measure with its history. None presents a sufficient fixity to constitute by itself the necessary and characteristic attributes. Agglutination by a specific serum ought not to be considered an exception to the rule, and there is no reason to think a priori that its power may be reduced below that of the common species, without the vibrio having lost at the same time its pathogenic properties for man.

The instructions issued by the minister of the interior of France to the laboratories charged with the final examination of suspected material after having stipulated as has been seen, further added that every vibrio agglutinable by the specific serum furnished in the proportion of 1 to 2,000, was to be considered as true cholera, and also stated the following reservation:

Attention has been drawn to the fact that a certain number of vibrios do not agglutinate at all, or agglutinate badly at the time when they have just been isolated from the human economy or from waters, and that they only acquire this property after a certain number of passages made daily upon artificial media. It would be well therefore, such a case arising, to repeat every day the agglutination experiments, and to defer considering as choleraic every vibrio isolated from a case presenting the clinical aspects of cholera and possessing the other characteristics so well known of the cholera vibrio.

So far as is known, instances of vibrios, classed at the time of their isolation as common species, which acquired later characteristics which obliged them to be considered as choleraic by reason of a high agglutinative power, have up to the last few years been very rare. According to the observations made in 1909 and 1910 at St. Petersburg, and the recent work of Zlatogaroff, they would be even less. This observer examined the stools of 1,540 persons, either convalescents from cholera or persons in good health, but who had been in contact with cholera, and found nonagglutinable vibrios 82 times. Of these 82 vibrios, 55 were subjected to various procedures, in con-

sequence of which 45 became agglutinable, and had to be considered as choleraic. Vibrios isolated from stools of cholera patients and from water have alike been rendered agglutinable. All these vibrios without exception were mono-ciliated, and presented otherwise the general characteristics of the cholera vibrio.

The observations of Zlatogaroff would lead to extending very much the limit of the reservation formulated in the French instructions, so far as concerns nonagglutinable vibrios. Nevertheless they might not justify a conclusion reaching beyond a suspicion of cholera as long as a vibrio with positive reaction had not been isolated. It must not be forgotten in fact that an abundant vibrionic flora may be encountered in diarrheal affections having no relation with cholera.

Observation has well established as a fact that vibrionic species are rarely met with, and then always in small number, in human feces of healthy individuals in temperate climates, even though they are in the course of a gastrointestinal condition. Such is not the case in hot or tropical countries. Observations made at Tor show that if the feces normally are in general practically free from vibrios, these are found frequently and often in great abundance in diarrheal feces; in other words, that one had always to deal with an ordinary diarrhea or a diarrhea attributable to a specific cause, such as dysentery.

In the course of researches made in 1905 and 1906 at the lazaretto of Tor, and of which mention must be made later, Gottschlich isolated in association with agglutinable vibrios some other species which did not agglutinate, and did not give the Pfeiffer reaction. There were 32 in 1905 and 16 in 1906. All these vibrios without exception were encountered in persons suffering from intestinal disorders, such as dysentery and enteritis, and their presence could often be recognized by the examination of a simple preparation.

In 1911 the same observer examined at Tor 1,160 pilgrims, and 31 times found cholera vibrios and 23 times nonspecific vibrios. The latter were found exclusively among individuals stricken with diarrhea or dysentery, never among choleraics. At the last session of the committee Dr. Ruffer thus summarized the impressions produced in his mind by the general results of his observations made in Egypt:

I must admit that I have been struck since I have been at El Tor by the number of patients arriving who present vibrios, even in the years when there is no cholera. Dr. Crendiropoulo, one of my assistants, has examined from this viewpoint the stools of many persons in Egypt at times when there was no cholera, and it is astonishing to see how often he has found vibrios. It appears that there may really be some relation of cause and effect between the vibrios and diarrhea.

From the experimental point of view Metchnikoff has been able to bring about the production of a grave choleriform diarrhea, and even a true attack of cholera, developing with all the classical symptomatology (the stools becoming rice water and the vibrios abundant) by the ingestion by a man of cultures of the vibrio of Versailles. Now this vibrio isolated by Sanarelli from the water of a fountain at Versailles, where all manifestations of cholera were wanting, is not agglutinable and can not be considered either by its origin or its characteristics as belonging to the order of cholera vibrio. Its ingestion also produced fatal cholera in the young rabbit. The same observations likewise apply to the vibrio of St. Cloud, likewise isolated by Sanarelli from the water of the Seine.

If these observations tend to make one think that numerous vibrionic species are capable of producing in man, under certain conditions, diarrheas more or less severe, simulating sometimes a genuine attack of cholera, there are no observations which would justify the assertion that these diarrheas might develop into an epidemic form, suggesting cholera, in the absence of choleraic vibrios with positive reactions.

As early as 1893 Ruffer had isolated from five cases of colitis and dysentery, without symptoms or lesions of cholera, several vibrios presenting all the characteristics as then known of cholera, including the Pfeiffer phenomenon.

In 1905 F. Gottschlich, upon the outbreak of a severe epidemic of dysentery, which broke out in the camp at Tor upon the return of the Moslem pilgrimage, examined systematically the intestinal contents of 107 deceased pilgrims. In no case could the death either on clinical or anatomical grounds be attributed to cholera, yet 6 presented vibrios having all the morphologic and cultural characteristics of cholera vibrios, agglutinating in dilutions of 1–500 to 1–2,000 with a specific serum, active in dilutions of 1–3,000; and also giving the Pfeiffer reaction. These vibrios are to-day known to science as the El Tor vibrios.

Similar researches made in 1906 upon the intestinal contents of 127 pilgrims stricken with various infections enabled the same observer to isolate two vibrios, presenting like those of the previous year the characters of agglutination and bacteriolysis, and in 1907 a similar vibrio was found in a pilgrim dying of cancer of the stomach. If one is restricted to the definition which has already been given of the cholera vibrio, the El Tor vibrios would be considered as all true cholera, but neither in 1897 nor in 1905, 1906, or 1907 had any cholera been disclosed during the course of the pilgrimage, either at Hedjaz or Tor.

Whatever may be the explanation of the presence of these vibrios in the intestines of pilgrims returning from the holy places, it remains nevertheless remarkable that they had not brought about any manifestations of cholera in the eminently receptive population of Hedjaz or the camp at Tor. Hence it was indicated to determine whether bacteriology might not furnish a means of distinguishing them and of assigning them to a place in the group of agglutinable vibrios.

The problem was shown to be particularly interesting from the very beginning, for the reason that these vibrios were found in fact to present in many respects a peculiar physiognomy. The question raised by the discovery of the El Tor vibrios has thus been the origin of quite a series of researches, which, if they have not led to a definite result, have at least enriched science by very interesting results. These reactions belong to three orders of phenomena, viz, hemolysis, deviation of complement, and the secretion of soluble toxines.

Hemolysis.—In his report of the operations of the first German cholera commission, published in 1887, Gaffky mentions as one of the properties of the cholera vibrio, that of dissolving the red blood corpuscles, and the description which he gives of the phenomenon shows that the solution of the blood corpuscles is produced by the secretion of a substance diffused outside of the microbial cells, a *hemolysin,* to give it the name which it has since received. Gaffky says:

When a gelatin to which blood has been added is used as a culture medium, the cholera vibrios destroy the red corpuscles, as is to be clearly observed on plate cultures, and this well beyond the limits in which is produced the liquefaction of the gelatin. Upon the plates, colored red by the blood, the colonies of vibrios appear each to be surrounded by colorless halos, of very striking appearance. Moreover it is to be remarked that this characteristic is not peculiar to the cholera vibrio, but belongs also to other organisms.

Kraus and his pupils had already undertaken the study of hemolysis as a diagnostic point, permitting the differentiation of vibrios of various species (9).

The El Tor vibrios are hemolytic. It had been previously considered that this was an important differential characteristic, for the researches made upon a number of vibrios, choleraic and noncholeraic, had led to the belief that a strong hemolytic power was the exception for agglutinating vibrios, while it was the rule for the others. It appears, however, according to recent discoveries, that the proportion of hemolytic species among agglutinating vibrios is greater than had been previously admitted. Huntemüller found 35 species out of 55 experiments when he preserved the plates under observation for six days; he found only six when the period of observation was limited to 24 hours. At St. Petersburg it was found that out of 642 cholera vibrios, newly isolated from feces and water, 406 gave the hemolytic reaction, clearly and strongly.

The hemolytic vibrios, even when strongly hemolytic, do not always lend themselves to the extraction of soluble hemolysins, though many of them have it.

These hemolysins have the character of an antigen; that is to say, that by injecting into animals filtered cultures which contain it the production of an antihemolytic serum is brought about.

The preparation of antihemolytic serums and the reciprocal experiments of hemolysin and serum have not as yet furnished grounds of differentiation between the various vibrionic species.

So far as the experiments have gone, the serum corresponding to a hemolysin is shown to be active against others.

The hemolytic power is shown to be of different strengths among the various species which possess it. For the same species it is susceptible of greater or less variation, varying with time and the vicissitudes to which the cultures have been exposed. Despite these contingencies, which indeed are not in excess of the biologic properties of microbes in general, it is a characteristic which merits being borne in mind.

Deviation of complement.—The property possessed by cells and cellular extracts, sensitized by an immune homologous serum, of immobilizing the alexin, or as it is said of deviating the complement (reaction of Bordet-Gengou), has furnished bacteriologists with a very fruitful method of diagnosis which had not as yet been applied to the identification of cholera vibrios (10).

Crendiropoulo has tried 42 vibrios from the point of view of the agglutination reactions according to Pfeiffer and Bordet-Gengou. He has also experimented upon their hemolytic powers.

These vibrios were divided into four groups, thus constituted:

	Reaction of agglutination.	Pfeiffer reaction.	Bordet-Gengou reaction.	Hemolysis.
Group I—12 vibrios	+	+	+	—
Group II—6 vibrios	+	+	—	+
Group III—14 vibrios	—	—	+	++
Group IV—10 vibrios	—	—	—	—

Among the vibrios neither reacting as to agglutination nor giving the Pfeiffer reaction, there are quite a number which are capable, however, of deviating complement in the presence of an anticholeraic serum. In the second place, the El Tor vibrios are clearly separated from the group of other agglutinable species. This conclusion as to the El Tor vibrios has, however, been controverted. Betsche and Kohn, who have tried the reaction with various vibrios in the presence of various serums, have arrived at the following conclusions:

An El Tor vibrio IV, with El Tor serum, reaction positive.
A cholera vibrio with El Tor serum, reaction positive.
A vibrio Metchnikoff with El Tor serum, reaction negative.
An El Tor vibrio with a cholera serum, reaction positive.

The same results are obtained if in place of the vibrio El Tor IV the vibrio El Tor V is tried.

Neufeld and Haendel are of the opinion that the El Tor vibrios react as truly choleraics.

These differences are due perhaps to differences of technique, but are sufficient in any event to cause reservations as regards the El Tor vibrios. As to the fact that there are vibrios capable of deviating complement, though they react negatively to agglutination and Pfeiffer reaction, we lack facts showing the practical significance of these results; upon this point the progress of science must be awaited.

The toxin of the vibrios.—The symptomatology of cholera is of the nature of a toxic disease, and Koch had already expressed the opinion of the existence of a poison secreted by the cholera vibrio. The proof of their toxic properties, and the differentiation of the specific poison which they elaborate, would furnish certainly the basis of a classification of vibrios, at once very natural and very useful for the determination of cholerigenic species. If science has not advanced in this direction it gives nevertheless some very interesting facts.

The experiments made in order to prove the existence in a culture of the Koch vibrio of a soluble toxin analogous to the toxins of diphtheria and tetanus, capable of playing the rôle of an antigen—that is to say, the injection of which into an animal brings about the formation of a specific antitoxin—were for a long time without result. In the presence of their lack of success, Pfeiffer had advanced the hypothesis that the choleraic poison was of a different nature, and had given, in order to designate it, the term endotoxin. The existence of true soluble toxins secreted by cholera vibrios was definitely put beyond doubt in 1896 by the work of Roux, Metchnikoff, and Salimbeni. The toxin of Roux, Metchnikoff, and Salimbeni is not materially altered by a boiling temperature; it loses its activity by exposure to the air, above all by exposure to light, but keeps well in sealed tubes. It is to be remembered that the endotoxin of Pfeiffer, obtained by

bacteriolysis and maceration of the microbial bodies, bears without notable diminution of strength a temperature of 60° C. for one hour.

Guinea pigs are the most sensitive animals, and with large doses death may be produced in a few minutes. "The effect is truly like that of lightning, especially if the injection is made into the abdominal cavity." The fall of temperature immediately follows the administration of the poison and continues until death. The animals vaccinated by injections of toxin or by intravenous injections of living cultures give an antitoxic serum.

Salimbeni on the one hand and Brau and Denier on the other have obtained toxins which appeared identical with each other and with the one just described, and have vaccinated animals and prepared antitoxic sera.

Kraus has shown that the filtered cultures of a certain vibrio (vibrio of Nasik) isolated in the Indies by Dr. Simon from an authentic case of cholera, but which is not to be regarded as truly choleraic, as anticholeraic serum does not agglutinate it (its homologous serum does not agglutinate cholera vibrios), contains a soluble toxin, the most striking characteristics of which are on the one hand its frailty, especially in the presence of elevated temperatures, on the other its property of killing rabbits in a few minutes by the intravenous route, and which also contains a soluble hemolysin. A short heating at 58° C. destroys both the toxin and the hemolysin. Animals immunized with it give a serum, which, mixed with the toxin in vitro, immediately neutralizes it.

Kraus and Pribram have shown that the vibrios of El Tor give a thermolabile toxin with a rapid action on rabbits, and a soluble hemolysin similar to that of the vibrio of· Nasik. The serum of animals vaccinated against the El Tor vibrios neutralizes the toxin of Nasik, and reciprocally, but only the serum of El Tor is active in the presence of a vibrio of Saigon, typically choleraic and nonhemolytic, which itself gives an immune serum, inactive against the two other toxins.

Reciprocal action of serums and toxins corresponding to the vibrios of Saigon, El Tor, and Nasik, according to Kraus.

Serums.	Toxins.		
	Saigon.	El Tor.	Nasik.
Saigon	+	−	−
El Tor	+	+	+
Nasik	−	+	+

Huntemüller, in a recent work, has shown that several choleraic vibrios are capable of producing toxins and hemolysins in every respect analogous to the products of the El Tor vibrios.

The serum of animals immunized against the toxin of one of these vibrios (vibrio 70 of the Berlin collection) is at the same time antitoxic and antihemolytic. It neutralizes equally the toxin of the El Tor vibrios. Moreover, animals immunized against the El Tor toxin give a serum which netralizes the toxin of vibrio 70.

All that it appears possible to deduce at the present time from these fragmentary facts would be the opinion that the poisons secreted by various cholera vibrios belong to two classes, represented on the

one hand by the endo-toxin of Pfeiffer, by the toxins of Roux, Metchnikoff, and Salimbeni, of Brau and Denier, or at least by the thermostable members of this group; on the other hand by the thermolabile toxins obtained from the vibrios (El Tor, vibrio 70) which all present the common characteristic of being strongly hemolytic.

The consideration of the vibrionic toxins and of their corresponding serums leads to conclusions as to the affinities which the ones exercise within the limits of the groups of the vibrios of positive reactions, but to which the others do not reach. The vibrios of El Tor answer on the one hand to certain true choleraic organisms (vib. Saigon and vib. 70); on the other hand to the group of hemolytic, but nonspecific vibrios (vib. Nasik).

From the preceding the definite conclusion may be drawn that considerations relative to the hemolytic power of vibrios, to their faculty of producing deviation of complement and of their toxins do not furnish at the present time such reactions as would permit the establishment between choleraic vibrios of differentiations of a practical character.

The fact that the bacillus carriers of the vibrios of El Tor did not plant cholera either at Hedjaz or at the lazaretto, signifies consequently that the discovery of agglutinable vibrios, hemolytic or not, and apart from all choleraic manifestations, should be interpreted with prudence, and not as constituting for the ensemble to which the carriers belong, a state of infection. This is the true meaning of the conclusions adopted by the committee as regards bacillus carriers. A very recent observation of Crendiropoulo and Madame Panayotatou lends support to this manner of thinking. These observers have isolated from the stools of a patient arriving from a Province of Egypt (where there have been no manifestations of cholera since 1902) at Alexandria, in order to receive treatment for a Billharzian diarrhea from which he had suffered for three years, two monociliated vibrios, agglutinable in the proportion the one of 1 to 2,000, the other in 1 to 500 by a serum active in 1 to 3,000, and giving both the reaction of Pfeiffer; the reaction of Bordet-Gengou positive in one and negative in the other, and neither hemolytic. In 22 days after the examination the vibrios had disappeared from the stools.

The serum of the patient agglutinated neither cholera vibrios, nor the vibrios isolated from his stools; it was not bacteriolytic and did not give the Pfeiffer reaction. It was therefore a question simply of a healthy bacillus carrier. The point of interest in the case is that he came from a neighborhood which had not for a long time been visited by cholera; that the most minute inquiry failed to show any communication, even presumptive, between him and cholera patients or persons who might themselves have been bacillus carriers. Moreover, though suffering from diarrhea, and consequently in a specially receptive condition, in spite of the presence of vibrios in his intestines of a choleraic nature, he did not himself have cholera.

III.

The research of the agglutinating power and of specific bacteriolysins in the blood of persons suspected to have undergone an attack of cholera permits the establishment of a diagnosis, even at a period

when the vibrios themselves have disappeared from the intestine. It would be sometimes possible then by this means to bring to light, up to the time of the first positive cases of an epidemic, incidents, and to thus establish the fact of unsuspected cases, establishing a relation between other patients and isolated cases.

In the researches made by Gaffky in 1905, out of 14 specimens of blood examined it was possible in 7 instances to affirm that there had been an attack of cholera, though the vibrio could not be discovered in the stools.

In 3 only of the 7 cases could indication be found as to the period of the disease at which the examinations were practiced. In an individual taken sick on August 28 five examinations made of feces from September 7 to October 8 were all negative. On September 10 his serum was agglutinating in the proportion of 1 to 500. In a second patient, taken sick on August 29 and pronounced cured on September 15, three examinations of feces, made on September 6, 15, and 23, were all negative; the serum, however, on September 21 gave the Pfeiffer reaction in 1 to 300, but only agglutinated in 1 to 50. In a third patient the first examination of the stools was only made on the day that he was pronounced cured. It was negative, as well as two others made in the space of nine days, but on the ninth day his serum gave the Pfeiffer reaction in dilution of 1 to 200, but was only agglutinable in 1 to 50.

The preceding shows that, from the point of view of the discovery of the specific antibodies, the Pfeiffer reaction is superior to that of agglutination. This, indeed, is the conclusion of Gaffky from the total of his observations. It is clearly shown in the table below, in which are found included the two results, side by side. In two other cases the positive result of the Pfeiffer reaction was obtained 23 and 24 days after the disease.

Agglutination.		Pfeiffer phenomenon.	
Dilution.	Result.	Dilution.	Result.
20	−	500	+
50	−	300	+
50	−	200	+
50	+	500	:+
100	−	250	+
50	+	500	+
100	−	500	+
100	+	500	+
200	−		
100	+		
200	−		
100	+		
200	−		

The facts collated by Svenson also confirm these observations. This observer having investigated the agglutinating power and the power of giving the Pfeiffer reaction in the blood of 27 cholera patients whose cases had been confirmed by the finding of the vibrios in their stools, found 21 positive results in the Pfeiffer experiment, while the agglutination was positive six times only in a dilution at most of 1 to 50.

Any agglutination not produced in a dilution greater than 1 to 50 should be regarded as negative. Jansen found once an agglutination in 1 to 640.

Gaffky found in the blood of three bacillus carriers specific antibodies in high dilutions.

Van Loghen examined in 1910 the feces of 298 healthy individuals composing the crews of 14 steamers coming from Russia to Rotterdam. He found four bacillus carriers, and investigated the bactericidal action of their serum. He employed a culture one loopful of which killed a guinea pig in 24 hours. While 50 milligrams of the serum of a normal man, and sometimes 250 milligrams, did not prevent the death of the guinea pig and did not produce the Pfeiffer reaction, 5 milligrams of the serum of the bacillus carriers sufficed to save the inoculated guinea pig and to produce the Pfeiffer phenomenon.

These facts which are explained easily by admitting that in bacillus carriers considered healthy there are produced true infections too slight to be noticed, but sufficient to produce the formation of bactericidal antibodies, show that even among healthy bacillus carriers a retrospective diagnosis is not always impossible.

EXPLANATORY NOTES.

(1)

The vibrio of cholera was described by Koch as an organism 0.5 micron broad, and three to four times as long, and very motile. When examined in suspension in a liquid, or in the hanging drop, it is seen running about the field in directions practically straight, and with a very lively movement. "Principally in the neighborhood of the margins of the preparation, where the vibrios are found in large numbers, they throw themselves about here and there in a lively confused manner, producing a very characteristic impression, which reminds one of the dance of the larvæ of mosquitoes." (Gaffky.)

It is well colored by all the aniline dyes, and is not stained by Gram. The color most generally employed is the carbolized fuchsin of Ziehl, diluted ten times with water. This even permits securing a sort of differential coloration, the vibrios in general taking a less vivid color than the greater part of the microorganisms which accompany them.

The vibrio of Koch possesses a single cilia at one of its extremities. This can be easily stained by the method of Giemsa.

In preparations most of the organisms appear slightly curved in the shape of a comma. Two organisms placed end to end may give, according to their individual shape, the appearance of a half circle, or of a letter S.

According to Gaffky: "In cultures it is not rare to find more than two bacilli placed end to end. In colored preparations they appear as undulating, more or less long, filaments; when examined in the living state it is easily demonstrated that they form beautiful spirals, having a very great resemblance to the spirochæte of relapsing fever."

It may thus be deduced that isolated vibrios represent fragments of a spiral, and that the more or less curved forms are the various aspects of their projection upon the plane of the slide.

Most frequently the vibrios which are met in cases of cholera show themselves under forms which, while not always those of typical vibrios, still answer closely to the preceding description, sometimes corresponding exactly, sometimes differing only in the breadth and length of their respective organisms, their degree of curvature, and the liveliness of their motion. But sometimes they differ completely, and constitute long, straight rods, or ovoid or round organisms of coccoid form. Motility, even, is not a constant characteristic, and may be completely wanting.

External morphological characteristics are also far from being immutable for each species, and the vibrios preserved in laboratories, and especially when they have been only recently introduced there, have a marked tendency to undergo morphological modifications, according to the influences to which they are subjected, such as the nature of the culture media, passage through animals, etc.

(2)

Dunbar reports the following experiment made in Hamburg in 1893. Preparations of fecal matter originating, some from individuals in whom, from the aggregate of clinical and bacteriological signs the diagnosis of cholera had been made, and some from others in whom it had been impossible to make a diagnosis, were mixed together, and submitted to the examination of two different bacteriologists. Out of 142 cholera preparations, one recognized 70, the other 59.

For the first, the detail of results, taken in connection with the clinical features of the case from which the preparations originated, were as follows:

Clinical diagnosis.	Microscopic diagnosis.	
	Positive.	Negative.
Cholera	41	27
Gastro-enteritis	25	22
Without symptoms of illness	4	23

(3)

The vibrio of Koch grows well upon all the media in current use in laboratories. It is essentially arobic. In liquid media it develops quickly and especially in the superficial layers, where it forms, conditions being favorable, thick and wrinkled veils or layers.

It does not grow in media even slightly acid, preferring a frankly alkaline reaction, and can support doses of alkali which arrest growth of a number of microbes with which it is found most frequently mixed in fecal matters, notably the *B. coli*. It is largely upon this last property that is based the composition of the elective media, of which subsequent mention will be made.

The proportion of alkali which affords the optimum of culture differs in various species. According to Dullman it is comprised in general terms between the extreme limits indicated in the table below, the figures representing the number of cubic centimeters of normal alkaline solution which must be added to 100 cubic centimeters of bouillon, assumed to be neutral to litmus.

	Solution of caustic soda.	Solution of sodium bicarbonate.
Vibrio of eastern Prussia	1.95	1.70
Vibrio of Witze	.78	.68

The dosage of alkali which it is necessary to employ to obtain media clearly differential for the cholera vibrio and the bacteria of feces (colon, typhoid, paratyphoid, dysentery) are much higher in the one case than in the other.

By reason of the formation of alkaline albuminates, in order to obtain equivalent results, doses of alkali are required, large in proportion as the media are rich in albumen, and after alkalinization the media must be more strongly heated.

In dilute peptone solution (1 to 100) at 37° C. the cholera vibrio develops rapidly, and even at the expiration of six hours gives an abundant culture. The greater part of the bacteria which ordinarily accompany it grow less quickly. With a bouillon of meat alone the results are less to the advantage of the vibrio.

Dunbar first conceived the idea of resorting systematically to preliminary cultures in peptone water for the search of the vibrio in pathological feces. These were planted in the average proportion of 0.1 cubic centimeter in 10 cubic centimeters of media. "Frequently, at the end of six hours a pure culture of vibrios might be collected from the surface and the immediate vicinity. In every case the culture even when mixed with other bacteria is found to be much enriched in vibrios, so that subsequent searches for the latter are found to be greatly facilitated. Experiments have shown that often in cases where isolations have been attempted on plates, starting with the original material and have failed, those starting with peptone water cultures have succeeded.

"The best time to utilize the cultures in peptone water is six hours after planting; sometimes it is necessary to wait longer. In every case it is necessary to practice

plants from time to time, in order to be able to grasp the moment when the vibrios predominate. Later the bacteria of feces also invade the culture to an extent not permitting the isolation of the vibrio in new plantings." (R. Koch.)

When one is dealing with materials containing very few vibrios, as is usually the case in those coming from bacillus carriers, it may sometimes be of value to make the first planting from the peptone water culture at the end of 3 hours in the incubator.

Sometimes the cultures made from the peptone water not having given results up to 12 hours, these may be obtained from later ones, up to 24 hours, but there appears no reason ever to pass this limit. Some bacteriologists prefer, in place of making successive plants from the same culture, to make a series of plants in peptone water, planting, for example, every 4 hours a new tube, with a sowing taken from the superficial layer of the preceding tube.

The German instructions of March 21, 1907, give the following formula for the preparation of the peptone water:

Dry Witte's peptone	grams..	100
Common salt	do....	100
Nitrate potassa	do....	1
Crystallized sodium carbonate	do....	1
Distilled water to make	liter..	1

This gives a mother solution, which for use is to be diluted with nine times its volume of water.

Metchnikoff has recommended a medium which contains, in addition to peptone and salt, a small quantity of gelatin. The formula is—

Dry peptone	grams..	1
Common salt	do....	0.5
Gelatin	do....	2
Distilled water	do....	100

made slightly alkaline with solution of soda.

Solutions of commercial peptones may with advantage be replaced by peptones prepared in the laboratory, as, for example, those prepared by the digestion of pigs' stomachs in a solution of HCl, 5 per 1,000.

When the vibrio is being sought in water, use is generally made of quantities of 100 to 1,000 c. c. In every case the water to be examined is collected in a sterile flask, and to it is added one-tenth of its volume of the ten times concentrated solution of peptone and salt, like the solutions of peptones employed for preliminary cultures. These are put in the incubator, and the steps are similar to those for ordinary plantings in peptone solution. If it should be desired to operate upon larger quantities of water, they are filtered through a bougie of porcelain, or better, through a bougie covered externally with a pellicle of collodion. The residue remaining upon the bougie is finally brushed off, diluted in a small quantity of sterile water, and the experiments are made with this mixture.

(4)

After 24 hours at 22° C. in the incubator the colonies already appear under the form of small, opalescent disks, with a slightly granular surface and wavy outlines. In one day more the disk has become thickened and extended; it is clear, and its granular and brilliant surface presents the appearance of a mass of small glass pearls; its borders are like lacework. Later it is surrounded by a zone of liquefaction; it forms a capsule full of a transparent liquid, at the bottom of which rests the colony. The liquefaction progresses slowly; it spreads for 5 or 6 days to the entire plate, which gives out a peculiar odor, compared to that of the "urine of mice."

Upon plates planted with pure cultures of cholera vibrios there are often found alongside of typical colonies answering to this description other colonies, cloudy, with irregular outlines, more or less active liquefaction, and having nothing characteristic in their appearance. When the plantings are made, starting with choleraic fecal matter (or from cultures in peptone water), the typical and atypical colonies may exist alongside of each other. Often the first predominate, but sometimes also they are entirely absent.

Finally, there exist, principally in waters, numerous vibrionic species which give on gelatin, colonies absolutely similar to typical choleraic colonies. These species are found, though generally rarely it is true, in human feces, in particular among persons who have partaken of waters in which they abound.

By reason of these peculiarities, the deductions which can be drawn from the appearance of colonies upon gelatin plates are somewhat misleading.

The same may be said of stab cultures in gelatin.

(5)

The medium of Dieudonné is prepared as follows: Equal parts of a normal solution of potassa and defribinated ox blood are mixed and sterilized in the autoclave (sol. A); there is also prepared according to the ordinary technique a nutrient agar, exactly neutral to litmus (sol. B). Seven parts of B are mixed with three parts of A and poured upon plates.

When the mixture of blood and alkali is heated a part of the latter is found to be saturated by the albuminoids or the products of their hydrolysis, but the final agar still preserves a very strong alkalinity, corresponding to about 0.6 per cent of potassa.

The free alkali and the alkaline combinations formed during the heating of the blood give together to the medium some special qualities. This does not happen with the nutrient agar prepared according to the ordinary technique and alkalinized to the same degree nor according to Huntemüller with an alkaline agar to which is added at one period the defribinated blood, prepared aseptically. They disappear also if, following the method of Dieudonné, a smaller quantity of potassa is put into a mixture of equal parts of blood and water.

The plates ought not to be used immediately after their preparation. Dieudonné recommends to keep them several days in the incubator at 37° C., uncovered and face down, or to heat them for five minutes at 65° C. An equally good result is obtained by keeping them for 48 hours at laboratory temperature. During this time the surface of the agar becomes slightly dry and loses a part of its alkalinity by the evaporation of the ammonia, formed in quite a large quantity by the heating of the alkaline blood. This is without doubt the reason of the advantage which this kind of maturation of the agar blood presents, which, above all, should not be pushed too far. Once in condition (by keeping for 48 hours at ordinary temperature, for example) the plates ought to be used within a period not exceeding five or six days.

The necessity of preparing plates quite a long time in advance or of heating them to 65° C. may in certain cases be annoying. Neufeld and Woithe claim that they obtain a medium, capable of immediate use (but not preserving its elective qualities for more than 24 to 48 hours), by adding to each 10 cubic centimeters of agar blood, 0.2 cubic centimeters of a 10 per cent solution of lactic acid.

Upon the agar of Dieudonné, planted in streaks, the cholera vibrios grew abundantly. On the contrary, the organisms which most often accompany them on plate cultures, and in the first place the *B. coli*, grow either very badly or not at all. The medium possesses in these two respects a very extremely marked power of election.

The typhoid bacillus, the paratyphoid bacilli, and the bacillus of dysentery deport themselves in general like the *B. coli*. On the contrary, the noncholeraic vibrios of water and of feces, the *B. proteus*, the *B. pyocyaneus*, and several other organisms behave very nearly like the cholera vibrio.

The *B. proteus*, which is encountered quite frequently in diarrheal stools, and the *B. pyocyaneus*, also very frequent in the diarrheas of hot countries, are known to complicate the search for cholera vibrios. In this respect the difficulty is not obviated.

The cholera colonies, developing upon the medium of Dieudonné in spite of their transparency, their grayish tint, and their glistening appearance by reflected light, are in fine only a little characteristic. When they are found mixed with a great number of others their search remains difficult and may present deceptive points. For this reason the employment of hyperalkaline blood agar, extremely valuable for bacteriological examinations to be essayed upon fecal matter, is much less so when it comes to hunting for cholera vibrios in the midst of the bacterial flora of waters.

The culture of the vibrios upon the medium of Dieudonné causes no sensible modification of the cultural or biological characteristics. Liquid media prepared by mixing in the proportion of 3 to 7, or of equal parts, of alkaline blood and neutral peptone water, present no special advantage for the culture of vibrios. Such media, which it has been tried to use for enrichment cultures, do no better than ordinary peptone salt solutions.

(6)

These are the directions given by Crendiropoulo and Madame Panayotatou: The medium is composed of peptone alone, but all of the commercial preparations do not appear equally adapted to its preparation. Among the products of the various manufacturers which have been tried, the peptones of Witte and Chapoteau alone have given satisfactory results. The method of preparation is as follows: Five grams of peptone are dissolved in 190 cubic centimeters of water; 10 cubic centimeters of a 10 per cent solution of caustic soda is added, and the mixture is heated for three to five minutes; after cooling it is filtered through paper and heated to 100° C. for a half hour. When the peptone of Witte is used it is preferable to diminish the dose of

soda, and to add only 8 cubic centimeters in place of the 10 cubic centimeters of the 10 per cent solution. It is very important not to depart from these proportions, because the combination of the soda with the peptone ought to be such that the liquid is left after sterilization of a degree of alkalinity comprised between 0.28 and 0.40 per 100, calculated as soda. At the moment of using, 4 parts of the alkaline peptone are mixed with 6 parts of neutral agar (agar 3 grams, peptone 1 gram, sodium chloride 0.5 gram, water 100 grams) and it is poured on to plates. The mixture should be made aseptically, because a further sterilization produces hydrolysis of the agar, which becomes brown and less fit for use. For planting, a certain small quantity of the material to be examined is diluted in a little normal salt solution, or preferably in peptone water; from this dilution 4 to 5 loops are touched to various parts of the plate and spread by means of a little glass rod.

In preliminary experiments made by planting simultaneously the cholera vibrio and the *B. pyocyaneus*, colonies of the vibrio alone were encountered at the end of 24 hours, to the exclusion of all others, while control cultures upon ordinary agar showed pyocyaneus almost exclusively, and very rarely a few accompanying vibrios. Experiments made with stools artificially infected, and with cholera stools, gave similar results.

(7)

Cultivated in peptone solutions (petone 1 per cent, NaCl 0.5 per cent) containing a small quantity of nitrates, the vibrio of Koch gives, at the expense of the former, some indol, and by reduction of the second, nitrites. The simultaneous presence of these two transformation products is shown by the red color which the liquid takes upon the addition of a few drops of a mineral acid, sulphuric acid free from nitrous products, for example. In the absence of nitrates in the medium, indol alone would be found in the culture, and might be put in evidence by the addition, after the mineral acid, of a few drops of a 0.5 per cent solution of sodium nitrite. All commercial peptones do not lend themselves equally to securing this reaction, but good results can be surely obtained by employing 1 per cent solutions of pepsic peptones, prepared in the laboratory by the digestion of pigs' stomachs in hydrochloric solutions, neutralized, and with the addition of 1 per 1,000 of nitrate of soda.

The experiment is only conclusive when carried out on a pure culture, and it is best not to make it until after 12 hours' stay in the incubator.

(8)

Agglutination reaction.—The agglutinative power of a serum, the titre of dilution (made in physiologic salt solution) is that dilution in which 1 c. c. agglutinates completely (the flakes deposited at the bottom of the test tube, leaving a clear liquid above) a definite quantity of culture (1 oese), under definite conditions (temperature 37° C.), in a given time (2 hours).

The normal serum of animals possesses the power in the presence of microbes in general, and of vibrios in particular, of agglutination, sometimes in a very marked degree. The existence of this common property might be a source of error, but it is avoided by using only immune serums, the specific activity of which is quite elevated. It is therefore indicated to resort to the immune sera obtained from animals in which this agglutinating power is least marked, when this course in practicable.

The order of preference of animals for the preparation of immune sera is, the rabbit, the ass, the goat, and the horse. For laboratory experiments which require the procuring of a serum corresponding to a given microbe, the rabbit remains the animal of election. When it is a question of securing large quantities of serum, practical considerations lead to the choice of the horse, and it is with horse serum that the work of laboratories is generally done.

The best method of procuring immunization is to inject by the intravenous route cultures killed by heating for one hour at 60° C. With rabbits three injections (1 oese, 3 oese, 5 oese), at intervals of 7 days, generally suffice to obtain by a bleeding made 7 days after the last injection a serum of very strong agglutinating power. (Kolle and Gotschlich.)

The agglutinating power of serums preserved in a liquid state may vary within very large limits. It remains more constant when the sera are dried in vacuo, and kept in sealed tubes, protected from the influence of light.

For the identification of vibrios it is well to employ only a cholera serum the agglutinating titre of which is 1–4000, or even higher.

Experiments are made (1) with a preliminary dilution in order to have a positive indication as to the character of colonies upon solid media, which it is desired to plant in order to secure pure cultures; (2) a definite, precise dilution, in order to determine

the exact value of the activity of a cholera serum in the presence of the given vibrio which it is desired to identify.

Preliminary experiment.—In order to secure a clear result in a short time it is convenient to employ quite strong doses of the serum. With a serum the activity of which is 1–5000 for example, the dilutions would be 1–500. The operation might be performed by distributing in the diluted serum a small quantity of culture taken from the suspected colony. In a few minutes, 30 or more, the drop should appear as if composed of a clear liquid in which the agglutinated microbes form a network, similar to the meshes of a fishnet. A control drop of physiologic salt solution, containing as nearly as possible the same quantity of culture, should preserve its uniformly cloudy aspect. This control is absolutely necessary for numerous microbial species, above all in very young colonies, spread very badly, and might produce appearances simulating an agglutination.

The phenomenon can be observed without difficulty with the naked eye, but can also be followed under the microscope, between slide and cover slip, under a low magnification.

If the agglutination shows itself under the conditions indicated, the colony is probably choleraic; if not, the experiment is to be regarded as inconclusive.

Determination of agglutinating power.—A series of dilutions of the immune serum in physiologic salt solution is prepared in the proportions of 1–200, 1–500, 1–1000, etc., and the liquid is filtered, if need be, to secure an absolutely clear liquid. Then 1 c. c. of the dilutions is put into small test tubes, and in each of them there is rubbed up, with careful shaking to obtain a uniform cloudiness, one loopful of a culture upon agar, 18 hours old, of the culture to be tested. These dilutions are put in the incubator at 37° C., and observed at the end of two hours. Every dilution is considered to have given a positive reaction, in which the vibrios are collected in flakes and deposited at the bottom of the tube, leaving a clear liquid above.

There should be made at the same time and under the same conditions two control observations—one with the culture to be identified, and the normal serum of an animal which has been used to furnish the immune serum, and another with the immune serum and an authentic cholera vibrio.

When it is desired to experiment with very young cultures (a few hours old only) there is sometimes produced even in physiologic salt solution without the addition of any serum, a pseudoagglutination. This inconvenience can be avoided by using only cultures which have been at least 15 hours in the incubator. If it is desired to use younger cultures, this incident should be provided against by a previous control experiment.

To be perfectly sure, the agglutination experiments should be made only upon pure cultures. When operating upon impure cultures of cholera vibrios, there may result no perceptible phenomenon, even under the microscope.

Experiments with the feces directly.—Dunbar has proposed, with a view to obtaining a rapid preliminary result, to make the agglutination experiments upon the suspected feces themselves. Upon two cover slips are deposited two drops, one of cholera serum in the dilution of 1–500; upon the other a drop of normal serum of the same species of animal, diluted 1–50; then in each of the drops is suspended a small quantity of the suspected material, and the cover slips are then inverted upon slides for microscopic examination.

When the material examined represents, as often happens, a pure or almost pure culture of cholera vibrios, on examining the two drops comparatively, it is seen that in the drop representing the normal serum there is a very abundant, actively motile flora, while in the other they are immobolized. The operation is rapidly done, not requiring in general more than 5 minutes, and the comparative aspect of the two drops is sometimes very characteristic. By prolonging the examination there may be observed in the immune serum drop a genuine agglutination. It is well to know that sometimes, even in the normal serum, the vibrios present a temporary immobilization. When the experiment is made upon material where vibrios are found mixed with an abundant and varied microbial flora, it becomes difficult, if not impossible, to count upon results of any value.

Culture in the presence of agglutinating serum.—Bandi has described as a rapid method of diagnosis the following procedure: A culture medium is made with a peptone and salt solution of 1 per cent to which is added anticholeraic serum in a proportion corresponding to about one-half of its agglutinating power. This medium is distributed in quantities of about 5 c. c. in small tubes, terminating at the base in a drawn-out point. They are planted on the surface with the material to be examined by means of a platinum loop, and put in the incubator at 37° C. If a culture, pure or nearly so, of cholera vibrios is under examination, these multiply and form flakes, which little by little accumulate in the drawn-out end. In a short time, say 2 to 7 hours, a very characteristic agglutinated culture may be thus secured. When dealing with material

containing a complex flora, the method may be practiced with the same observations as to reliability as the one above.

REACTION OF PFEIFFER.—The reaction of Pfeiffer may be tried in two ways, *in vivo* according to the method of Pfeiffer or *in vitro* according to the method of Bordet.

The bacteriolytic power of a serum is defined as that dilution, made in meat bouillon, of which 1 c. c., to which is added one loop of an 18-hour old culture on agar, virulent and kept for 18 hours in the incubator at 37° C., injected into the peritoneum of a guinea pig, brings about in one hour, after a transformation, the dissolution of nearly all the vibrios.

Pfeiffer's method.—It is preferable to employ as immune serum that of the rabbit, for the normal serum of other species of laboratory animals (the horse, the ass, the goat) is generally more rich in bacteriolytic substances, which, having no specificity, might be a source of error. To eliminate moreover as much as possible this latter chance it is best to use only serums having a high bacteriolytic power, 1—5000 at least.

According to Kolle and Gotschlich such serums are easily obtained by injecting at once into the peritoneal cavity of a rabbit an entire culture upon an agar tube killed by a heating for 1 hour at a temperature of 56° C. The blood is to be collected 14 days after, a rabbit giving on an average 40 c. c. of serum. The experiments ought to be made on young guinea pigs weighing about 200 grams. In 1 c. c. of a macera tion of meat one loopful of the culture to be tried is diluted, the culture being virulent, and 18 hours old, cultivated upon agar in the incubator at 37° C. There is then added a quantity of anticholeraic serum representing 5 times the dose of the limit of agglutinating power (that is to say 1 milligram of serum active at 1—5000, and this is injected into the peritoneal cavity of a guinea pig (A).

The injection is made after incision of the skin by means of a needle with a blunted point, which easily penetrates the abdominal walls, and which may be plunged without danger into the peritoneum. The removal of fluid from the peritoneum, with a view to examination, is made at the same point by means of a drawn-out glass pipette.

At the same time as the first guinea pig a second guinea pig is inoculated under the same conditions (B) with a loopful of the same culture diluted in 1 c. c. of mace ration of meat, but without serum, and a third guinea pig (C) with a similar dilution, to which is added a quantity of normal serum equal to 10 times the quantity of anti choleraic serum given to guinea pig (A).

The extractions of peritoneal fluid ought to be made at the end of 20 minutes and 1 hour, and these fluids should be examined in hanging drop, with a strong magnifi cation.

The reaction is positive if at the end of 20 minutes, or of 1 hour at the most, it is observed that in the peritoneum of guinea pig (A) the vibrios are dissolved, and have undergone the transformation into typical granules, while in the peritoneum of guinea pigs B and C the vibrios are found in great number, motile, and their forms well preserved.

In order that the experiment may be profitably made with a given vibrio, it is indis pensable that it should be virulent by intraperitoneal injection for a guinea pig, other wise the transformation into granules and the dissolution are produced equally in the control B, and the experiment is deprived of all significance.

To determine old cases of cholera, the Pfeiffer experiment ought to be made in the following manner, in accordance with the terms of the German instructions of March 22, 1907:

"Dilutions of the serum of the suspected person are prepared in meat maceration in the proportions of 1–20, 1–100, and 1–500, and with 1 c. c. of each of them is mixed a loopful of virulent 18-hour-old cultures, and the mixtures are injected into the peri toneal cavities of guinea pigs of about 200 grams weight. A control animal receives in the peritoneal cavity a loopful of the same culture, diluted in the same way in a centi meter cube of meat maceration, but without serum. If the reaction is positive at the end of 20 to 60 minutes, it must be admitted that the person supplying the serum has undergone an attack of cholera."

Method of Bordet.—The execution of the Pfeiffer reaction requires special conditions, notably as regards animals, which are not always to be met with, especially in labora tories of exigency, established to meet the demands of epidemics of cholera. Moreover the vibrios isolated in suspected cases, all or at least most of them present the degree of virulence necessary. For this reason a resort to the technique of Bordet appears to be quite frequently indicated. It is applied in the following manner:

A series of dilutions of the serum is prepared, commencing with a mother dilution of 1 to 50. Then there is poured into a series of tubes 5 drops of the guinea-pig serum (alexine), 5 drops of a microbial emulsion, made in the proportion of one loop ful of an 18-hour-old culture on agar in 1 c. c. of physiological salt solution, and the

number of drops of the mother liquid necessary to obtain the desired dilution, and enough of physiological salt solution to make 20 drops. A series of controls is made with the same quantities of microbes, alexine, and normal serum in place of immune serum. Crendiropoulo and A. Panayotatou recommend further, in order to avoid any doubt in the interpretation of results, the following series of control experiments, made by always putting in the same total quantity of fluid the same quantities of microbes and—

(1) In a tube the greatest quantity of the specific serum employed, without alexin.
(2) In a tube the greatest quantity of normal serum employed, without alexin.
(3) In a tube the quantity of alexin employed, without serum of any kind.
(4) In a tube physiologic salt solution alone.

The dilutions of serum ordinarily employed are 1–100, 1–500, and 1–1000. They are all put into the incubator at 37° C. and examined in hanging drop after 3 to 4 hours.

After 18 hours in all cases, where cholera microbes are being dealt with, it is seen in all the control mixtures without exception that the vibrios exist in abundance and living, while in the tubes of specific serum all more or less present only vibrios which have very completely undergone transformation.

(9)

Practically the investigation of the hemolytic power of vibrios is made on solid media, according to the method of Kraus and in liquid media, the culture and the blood corpuscles being diluted in physiologic salt solution.

Method on solid media.—Tubes of agar-agar are liquefied, and their temperature allowed to fall to 40° C. There is then added 0.3 to 0.5 cubic centimeter of defibrinated sheep's or goat's blood. A mixture is affected, and poured on to plates, which are then inoculated on the surface with the vibrio under investigation. After 24 hours in the incubator, if the vibrio is hemolytic, colonies are seen surrounded by an aureole, more or less extended, contrasting with the dark, opaque color of the rest of the plate.

Kraus recommends to limit one's self to the results obtained in 24 hours. Other observers prolong the experiment further, and consider the trial positive only at the end of two, four, or even six days. The results are very different, according to the method adopted. With the second method many more hemolytic species are found than with the first. But it is to be feared that when the plates remain several days in the incubator, the alkalinization of the medium by the ammonia produced in the culture, may have the effect of itself producing hemolysis.

Method in liquid media.—An 18-hour-old culture on agar is diluted with 4 to 5 cubic centimeters of normal salt solution; one-tenth cubic centimeter of this emulsion is added to a large drop of washed red corpuscles or simply a drop of defibrinated blood. The blood of the rabbit, the ox, the sheep, or the guinea pig is indifferently used.

The reaction may be carried on at the temperature of the room or in the incubator, but the results are not always the same in the two cases. When the experiment is made at room temperature, as a general rule the hemolysis is manifest in 12 to 18 hours. Sometimes it is delayed and appears more slowly; but there is no advantage in prolonging the observation beyond 48 hours.

(10)

The serum of an animal suitably immunized against a microbe exercises upon this microbe a bacteriolytic power. In the same way the serum of an animal into which have been injected red blood corpuscles taken from a different species acquires the power of producing a dissolution of these corpuscles. The bacteriolytic, as well as the hemolytic action of these two substances, one of which, the alexin or complement (which is completely destroyed by a heating of 56° C. for one-half hour), is common to all serums, while the other, the sensitizer or antibody (which is not destroyed by a temperature of 56° C. for a half hour) is specific and only appears in a serum as the result of appropriate treatment by the microbes or the red cells.

Microbes or blood cells suspended in liquid which contains their specific antibody, fix it, and remove it from the liquid. Thus sensitized, they have acquired the power of being acted upon by the alexin. This action of the former is shown in the case of the red cells by their solution and the setting free of their hematin, which colors the liquid red, thus rendering the observation of the phenomenon easy.

When a suitable quantity of sensitized microbes are diluted in a solution of alexin, the active substance is fixed by them, and the addition of sensitized blood cells gives rise to no hemolysis. It may, therefore, be tested whether a given vibrio presents the properties of an antigen in the presence of the antibody contained in a cholera

serum prepared by starting with a cholera vibrio. It is sufficient to try if this vibrio treated by a serum (previously heated for a half hour to 56° C. in order to destroy the alexin) is capable of fixing the alexin—that is to say, of preventing the hæmolysis of sensibilized red cells. The reaction may be tried according to the original method of Bordet. Serums without alexins are prepared by heating them for a half hour at 56° C.; on the one hand, an immune serum; on the other, fresh, normal serum of the same species; for example, the serum of the horse or the rabbit. It is well to employ an immune serum of high titre, say, 1–4,000 or more. There is employed as alexin, fresh serum of the rabbit or guinea pig; as the microbial emulsion, that obtained by diluting an entire agar culture, 18 hours old, in 2 c. c. of physiologic salt solution; as the hemolytic medium, a mixture of one volume of the red cells of the sheep (washed) and two volumes of rabbit antisheep serum (the serum of a rabbit treated by injections into the peritoneum ot the defribrinated blood or red corpuscles of a sheep) heated for one-half hour at 56° C. Each test consists in making use of the following mixtures:

	Tubes.		
	I	II	III
Microbial emulsion	0.2	0.2	0.2
Heated serum:			
Immune	.6	.2	.6
Normal	.6	.6	.6
Alexin	.1	.1	.1

These are kept for four to five hours at laboratory temperature, shaking from time to time, then there is added to Tubes I and II, one drop of the dilution of red cells in heated specific serum; Tube III, one drop of the dilution of red cells in physiologic salt solution.

The tubes are examined after three hours' stay at ordinary temperatures. If there is a deviation of complement, there is observed in Tube I, no hemolysis; Tube II, hemolysis; Tube III, no hemolysis.

When the microbe submitted to experiment is in itself hemolytic, the hemolysis appears in Tubes I and II, but more slowly, in 12 to 18 hours, so that it does not interfere with the execution of the experiment. The statements given relative to the quantity of alexin, of serum, etc., to be used should only be taken as a general indication. All fresh serums have not the same richness in alexin; nor all agar cultures the same richness in vibrios, so that in order that the fixation of the alexin may be complete, it is necessary that there should be a certain relation between the quantity of the former and the quantity of sensitized cells. Such an experiment, made with a certain quantity of alexin, might lead to the consideration as hemolytic any vibrio giving a positive reaction, while another made with a stronger dose might lead to a contrary conclusion. Neither is the richness of the hemolytic serum in antibody to be neglected.

In order to avoid as much as possible the sources of error, it would be well to determine the quantities of the various elements which give a clear phenomenon, by making a preliminary experiment with a cholera vibrio, and then to proceed to comparisons. In any event it is well to only consider as comparable such experiments as are made under rigorously identical conditions. Besche and Kohn recommend the employment for fixing the alexin, not the microbial body, but an extract prepared by commencing with a culture of vibrios in a Kolle flask, grown for 18 hours and emulsified in 15 c. c. of distilled water, heated for an hour to 60° C., shaking for a day at room temperature, and then centrifuging. In current practice, in order to obtain a result in three to four hours it may be sufficient to use in place of a microbial extract, an emulsion of one loopful of an agar culture in 2 c. c. of physiologic salt solution, heated for one hour at 60° C.

UNITED STATES.

CEREBROSPINAL MENINGITIS IN TEXAS.

Surg. Guiteras at Galveston reports 9 cases of cerebrospinal meningitis with 4 deaths at Galveston from March 1 to 4, inclusive

The health officer at Dallas, Tex., reported cerebrospinal meningitis present in Dallas as follows: 1911.—Month of October, 1 case; month of November, 9 cases with 6 deaths; month of December, 73 cases with 32 deaths. 1912.—Month of January, 221 cases with 85 deaths; month of February, 106 cases with 50 deaths.

At Fort Worth the health officer reports the occurrence of 75 cases with 32 deaths during the month of February, 1912.

At San Antonio the health officer reports 12 cases with 4 deaths for the month of February, 1912.

TYPHOID FEVER EPIDEMIC AT ROCKFORD, ILL.

The health officer reports typhoid fever present in epidemic form at Rockford, Ill., with 119 cases reported during the week ended February 17, and 170 cases during the week ended February 24.

PLAGUE-PREVENTION WORK.

DISTRIBUTION OF POISON.

In connection with the making and maintenance of a squirrel-free zone around the cities of California on San Francisco Bay, 180 acres of land in Alameda County were covered with poison during the week ended February 24, 1912.

During the same period 3,220 acres of land in San Joaquin County and 3,400 acres in Stanislaus County were covered with poison for the purpose of eradicating plague foci.

<div align="center">RECORD OF PLAGUE INFECTION.</div>

Places.	Date of last case of human plague.	Date of last case of rat plague.	Date of last case of squirrel plague.	Total number of rodents found infected since May, 1907.
California:				
Cities—				
San Francisco	Jan. 30, 1908	Oct. 23, 1908	None	398 rats.
Oakland	Aug. 9, 1911	Dec. 1, 1908	....do	126 rats.
Berkeley	Aug. 27, 1907	None	....do	None.
Los Angeles	Aug. 11, 1908	....do	Aug. 21, 1908	1 squirrel.
Counties—				
Alameda (exclusive of Oakland and Berkeley).	Sept. 26, 1909	Wood rat, Oct. 17, 1909.	Oct. 9, 1911	114 squirrels and 1 wood rat.
Contra Costa	July 21, 1911	None	Sept. 23, 1911	364 squirrels.
Fresno	None	....do	Oct. 27, 1911	1 squirrel.
Merced	....do	....do	July 13, 1911	5 squirrels.
Monterey	....do	....do	Aug. 6, 1911	Do.
San Benito	June 5, 1910	....do	June 8, 1911	22 squirrels.
San Joaquin	Sept. 18, 1911	....do	Aug. 26, 1911	18 squirrels.
San Luis Obispo	None	....do	Jan. 29, 1910	1 squirrel.
Santa Clara	Aug. 23, 1910	....do	Oct. 5, 1910	23 squirrels.
Santa Cruz	None	....do	May 17, 1910	3 squirrels.
Stanislaus	....do	....do	June 2, 1911	13 squirrels.
Washington:				
City—				
Seattle	Oct. 30, 1907	Sept. 21, 1911	None	25 rats.

<div align="center">RATS COLLECTED AND EXAMINED FOR PLAGUE INFECTION.</div>

Places.	Week ended—	Found dead.	Total collected.	Examined.	Found infected.
California:					
Cities—					
Berkeley	Feb. 24, 1912	5	[1] 148	85	....
Oakland	....do	13	[2] 563	382	....
San Francisco	....do	8	[3] 1,309	998	....
County—					
San Joaquin	....do	....	[4] 166	166	....
Washington:					
City—					
Seattle	....do	....	967	917	....

[1] Identified: Mus norvegicus, 95; mus musculus, 53.
[2] Identified: Mus norvegicus, 455; mus musculus, 105; mus rattus, 2; mus alexandrinus, 1.
[3] Identified: Mus norvegicus, 658; mus musculus, 323; mus rattus, 160; mus alexandrinus, 168.
[4] Identified: Mus norvegicus, 157; mus musculus, 3; mus rattus, 4; mus alexandrinus, 2.

SMALLPOX IN THE UNITED STATES.

In the following table the States indicated by an asterisk are those from which reports of smallpox are received only from certain city, and in some cases county, boards of health. In these States, therefore, the recorded cases and deaths should not be taken as showing the general prevalence of the disease. In the States not marked by an asterisk the reports are received monthly from the State boards of health, and include all cases reported to the State authorities.

SMALLPOX IN THE UNITED STATES—Continued.

REPORTS RECEIVED DURING WEEK ENDED MAR. 15, 1912.

Places.	Date.	Cases.	Deaths.	Remarks.
Alabama:				
Mobile	Jan. 1–Mar. 2	157		Year 1911; cases, 312.
Arizona	Dec. 1–31			No case.
California:				
Counties—				
Contra Costa	Jan. 1–31	2		
Fresno	....do	53		
Los Angeles	....do	29	1	
Riverside	....do	18		
San Bernardino	....do	34		
San Diego	....do	2		
Stanislaus	....do	1		
Total for State		139	1	
Colorado:				
Counties—				
Archuleta	Feb. 1–29	2		
Denver	....do	1		
Larimer	....do	1		
Mesa	....do	2		
Montrose	....do	6		
Rio Grande	....do	6		
Total for State		18		
Connecticut:				
County—				
Windham	Feb. 1–29	1		
Iowa:				
Counties—				
Carroll	Feb. 1–29	1		
Floyd	....do	1		
Harrison	....do	1		
Ida	....do	1		
Linn	....do	2		
Louisa	....do	1		
Marshall	....do	1		
Pottawattamie	....do	3		
Sac	....do	6		
Scott	....do	11		
Sioux	....do	5		
Washington	....do	1		
Woodbury	....do	3		
Total for State		37		
Kansas:				
Counties—				
Coffey	Jan. 1–31	4		
Crawford	....do	2		
Leavenworth	....do	5		
Lyon	....do	1		
Osborne	....do	7		
Riley	....do	2		
Shawnee	....do	1	1	
Woodson	....do	23		
Wyandotte	....do	4		
Total for State		49	1	
Massachusetts:				
Counties—				
Bristol	Feb. 1–29	9		
Middlesex	....do	4		
Suffolk	....do	1		
Total for State		14		
Missouri:				
St. Louis	Feb. 26–Mar. 2	5		
Nebraska:				
Omaha	....do	1		
New Jersey:				
County—				
Essex	Feb. 1–29	1		

SMALLPOX IN THE UNITED STATES—Continued.

Reports Received during Week ended Mar. 15, 1912.

Places.	Date.	Cases.	Deaths.	Remarks.
North Carolina:				
Counties—				
Beaufort	Feb. 1–29	2		
Bertie	....do	2		
Buncombe	....do	2		
Columbus	....do	2		
Cumberland	....do	28		
Forsyth	....do	1		
Gaston	....do	5		
Granville	....do	7		
Harnett	....do	1		
Haywood	....do	57		
Iredell	....do	10		
Jackson	....do	20		
Johnston	....do	1		
Macon	....do	1		
Madison	....do	14		
Mecklenburg	....do	3		
New Hanover	....do	3		
Orange	....do	5		
Pender	....do	3		
Sampson	....do	6		
Scotland	....do	2		
Swain	....do	20		
Union	....do	70		
Yancey	....do	3		
Total for State		268		
Ohio:				
Counties—				
Athens	Feb. 1–29	3		
Defiance	....do	1		
Hamilton	....do	8		
Lucas	....do	9		
Montgomery	....do	6		
Tuscarawas	....do	1		
Total for State		28		
Pennsylvania	Dec. 1–31		1	
	Jan. 1–31	15		
Total for State		15	1	
Tennessee:				
County—				
Shelby	Feb. 1–29	6		
Wisconsin:				
Counties—				
Adams	....do	6		
Brown	....do	4		
Dane	....do	1		
Douglas	....do	4		
Dunn	....do	1		
Eau Claire	....do	3		
Fond du Lac	....do	2		
Grant	....do	1		
Green	....do	1		
Green Lake	....do	3		
Jackson	....do	8		
La Crosse	....do	2		
Marathon	....do	1		
Monroe	....do	1		
Pierce	....do	1		
Portage	....do	25		
St. Croix	....do	5		
Trempealeau	....do	4		
Waupaca	....do	2		
Waushara	....do	7		
Wood	....do	12		
Total for State		94		
Wyoming:				
Counties—				
Albany	Jan. 1–31	4		
Larimer	....do	3		
Sweetwater	....do	11		
Uinta	....do	55		
Total for State		73		

SMALLPOX IN THE UNITED STATES—Continued.

Reports Received during Week ended Mar. 15, 1912.

Places.	Date.	Cases.	Deaths.	Remarks.
Wyoming—Continued. Counties—Continued.				
Albany	Feb. 1-29	2		
Carbon	do	3		
Fremont	do	8	1	
Laramie	do	3		
Sweetwater	do	6		
Uinta	do	15		
Total for State		37	1	
Grand total for the United States.		943	4	

For reports received from July 1 to December 29, see Public Health Reports for December 29, 1911. The cumulative table of reported cases of smallpox, heretofore published each week, has been discontinued, and in its place summaries will be published periodically.

MORBIDITY AND MORTALITY.

MORBIDITY AND MORTALITY TABLE, CITIES OF THE UNITED STATES, FOR WEEK ENDED FEB. 24, 1912.

Cities.	Population, United States census, 1910.	Total deaths from all causes.	Diphtheria. Cases.	Diphtheria. Deaths.	Measles. Cases.	Measles. Deaths.	Scarlet fever. Cases.	Scarlet fever. Deaths.	Smallpox. Cases.	Smallpox. Deaths.	Tuberculosis. Cases.	Tuberculosis. Deaths.	Typhoid fever. Cases.	Typhoid fever. Deaths.
Cities having over 500,000 inhabitants.														
Baltimore, Md	558,485	219	12	2	11		17	1			37	38	6	2
Boston, Mass	670,585	255	43	1	147	1	24	4			66	25	3	1
Chicago, Ill	2,185,283	749	100	10	98	2	190	12			220	27	18	1
Cleveland, Ohio	560,663	166	24	3	43	1	42	1			25	12	2	
New York, N. Y	4,766,883	1,635	338	25	1,289	18	387	18			479	220	26	8
Philadelphia, Pa	1,549,008	546	73	7	11		55	1			70	54	24	5
Pittsburgh, Pa	533,905	187	19	4	11	1	17		1					
St. Louis, Mo	687,029	238	20	3	18		29	4	1		48	22	3	
Cities having from 300,000 to 500,000 inhabitants.														
Buffalo, N. Y	423,715	89	23	1	40		26				21	9		
Cincinnati, Ohio	364,463	185	6		6		26	3	1		47	30	1	
Detroit, Mich	465,766	167	18				27							
Los Angeles, Cal	319,198	113	18		4		17		2		13	20	3	
Milwaukee, Wis	373,857	119	11	4	31		41	1			18	8	21	3
Newark, N. J	347,469	98	21	1		1	23				26	14		
New Orleans, La	339,075	183	5	2	4		10		6		26	22	2	2
San Francisco, Cal	416,912	150	1		253	3	3				13	14	4	1
Washington, D. C	331,069	134	9		3		7				31	11	2	
Cities having from 200,000 to 300,000 inhabitants.														
Jersey City, N. J	267,779	76										9		
Kansas City, Mo	248,381	43	7		6		7		3		8	15	3	1
Providence, R. I	224,326	74	19	8	13		9	1				5	1	
Seattle, Wash	237,194	32	5		11						3	2	5	
Cities having from 100,000 to 200,000 inhabitants.														
Bridgeport, Conn	102,054	33	5	1	3		9				6			
Cambridge, Mass	104,839	30	7	1	2		6				4	2	1	
Columbus, Ohio	181,548	71	3	1	46	1	18				7	7	1	1
Dayton, Ohio	116,577	40			1		2		2			5	1	3

MORBIDITY AND MORTALITY—Continued.

Morbidity and mortality table, cities of the United States, for week ended Feb. 24, 1912—
Continued.

Cities.	Population, United States census, 1910.	Total deaths from all causes.	Diphtheria.		Measles.		Scarlet fever.		Small-pox.		Tuberculosis.		Typhoid fever.	
			Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.
Cities having from 100,000 to 200,000 inhabitants—Con.														
Fall River, Mass	119,295	44	5	1	3		3				2	7		1
Grand Rapids, Mich	112,571	33					4				4	4	4	
Lowell, Mass	106,294	41	2		27	1	3				4	1		
Nashville, Tenn	110,364	41					2				4	3	2	
Oakland, Cal	150,174	43	8		1		3				1	4		
Omaha, Nebr	124,096	25	2				2					1		
Richmond, Va	127,628	44	5		14						7	2		
Spokane, Wash	104,402		1		10		3		13		2	2	2	
Toledo, Ohio	168,497	66	3		11		4		1			5	1	1
Worcester, Mass	145,986	51	10	1			8				7	1		
Cities having from 50,000 to 100,000 inhabitants.														
Altoona, Pa	52,127	26	2									2		
Bayonne, N. J	55,545	17	1		1		4	1			2	3		
Brockton, Mass	56,878	18	1		76		1				1	1		
Camden, N. J	94,538	12	1				3				5		3	
Duluth, Minn	78,466	14					5		2		2	2		
Elizabeth, N. J	73,409	32	5	2	2	2	7				1	2	1	1
Erie, Pa	66,525	17	6								6		3	
Evansville, Ind	69,647	16	4								2	4	6	2
Fort Wayne, Ind	63,933	17	2		3							1		
Harrisburg, Pa	64,186	28	4		23		1				1	1	2	2
Hartford, Conn	98,915		7	1			4				7	3		
Hoboken, N. J	70,324	9	2		1		4				2			
Johnstown, Pa	55,482	22	2				3					2		
Kansas City, Kans	82,331		1		1		1		2		5		9	
Lawrence, Mass	85,892	20	2	1			7				1	2		
Lynn, Mass	89,336	31	1		3		6	1			6		1	
Manchester, N. H	70,063	26	8	1					4		2	2		
New Bedford, Mass	96,652	32			22		6				10	3	4	
Oklahoma City, Okla	64,205	21	1				1				2	3		
Passaic, N. J	54,773	22	1		4		1					3		
Pawtucket, R. I	51,622													
Reading, Pa	96,071	27	9		3		2				2	2		
Saginaw, Mich	50,510		4	1			2						6	1
San Antonio, Tex	96,614	40			30		3				7	1		
Schenectady, N. Y	72,826	16	1		14		2				7	2		
South Bend, Ind	53,684	20							1			2	3	
Springfield, Ill	51,678	27	5				1		1			2		1
Springfield, Mass	88,926	29	2		23		3				2	2	1	
Trenton, N. J	96,815	36	1				5				7	5	1	1
Wilkes-Barre, Pa	67,105	26	2	1	21		2				7	1		
Yonkers, N. Y	79,803	17	3		10		10				3	3		
Cities having from 25,000 to 50,000 inhabitants.														
Atlantic City, N. J	46,150	4	1											
Aurora, Ill	29,807	9	1				4							
Binghamton, N. Y	48,443	20			1		2					1		
Brookline, Mass	27,792	6	1		1		1		1					
Butte, Mont	39,165	12			1		3				1			
Chattanooga, Tenn	44,604						2	1			2			
Chelsea, Mass	32,452	18	1		4		1				1			
Chicopee, Mass	25,401	5			1						2	1		
Danville, Ill	27,871	4												
East Orange, N. J	34,371	6	1				2				1			
Elmira, N. Y	37,176	9	2	1										
El Paso, Tex	39,279	31							4	2	3			
Everett, Mass	33,484	15	2				2				1			
Fitchburg, Mass	37,826	11	1								2			
Haverhill, Mass	44,115	17	3		13						3		1	
Kalamazoo, Mich	39,437	23					11	2			2	1	1	1
Knoxville, Tenn	36,346	11			2				11		2	2		
La Crosse, Wis	30,417	3	2				1							
Lancaster, Pa	47,227		1		11		1						1	
Lexington, Ky	35,099	16			3		3				2	2		
Lima, Ohio	30,508	8	2		1						1			

MORBIDITY AND MORTALITY—Continued.

Morbidity and mortality table, cities of the United States, for week ended Feb. 24, 1912—
Continued.

Cities.	Population, United States census, 1910.	Total deaths from all causes.	Diphtheria. Cases.	Deaths.	Measles. Cases.	Deaths.	Scarlet fever. Cases.	Deaths.	Small-pox. Cases.	Deaths.	Tuberculosis. Cases.	Deaths.	Typhoid fever. Cases.	Deaths.
Cities having from 25,000 to 50,000 inhabitants—Con.														
Lynchburg, Va	29,494	13	2		25		2				1	1		
Montgomery, Ala	38,136	16	2		21		1		1		2	2	1	
Mount Vernon, N. Y	30,919		7		28						1			
Newcastle, Pa	36,280		7				1				1		8	
Newport, Ky	30,309	11	3				3				1	1	1	1
Newton, Mass	39,806	11	1		16		2				2		1	
Niagara Falls, N. Y	30,445	12	3				2					1	9	
Norristown, Pa	27,875	9	2		57	2	1					2		
Orange, N. J	29,630	10	1	1	3		5							
Pasadena, Cal	30,291	13	2								1	2		
Pittsfield, Mass	32,121	9	3								1	1		
Portsmouth, Va	33,190	7	3		1				2					
Racine, Wis	38,002	10	7											
Roanoke, Va	34,874	6	1		28									
Rockford, Ill	45,401	15					1					2	¹170	3
Salem, Mass	43,697	13					1					1		
San Diego, Cal	39,578		1		2		1				6	6		
South Omaha, Nebr	26,259	7	1											
Superior, Wis	40,384	6							2					
Taunton, Mass	34,259	15			3						1			
Waltham, Mass	27,834	11	2		9							1		
West Hoboken, N. J	35,403	9	3		2		1				1	2		
Wheeling, W. Va	41,641	9	2				1				1	1	1	
Williamsport, Pa	31,860	7					4					2		
Wilmington, N. C	25,748	14			5		1		3		2	3	1	
York, Pa	44,750				1		2				2			
Zanesville, Ohio	28,026	9	1									4	3	1
Cities having less than 25,000 inhabitants.														
Alameda, Cal	23,383	7			1						2			
Ann Arbor, Mich	14,817	7												
Beaver Falls, N. Y	12,191	0					3							
Bennington, Vt		6					2							
Braddock, Pa	19,957											1		
Butler, Pa	20,782	10	1				1				1			
Cambridge, Ohio	11,327	3			4		1							
Camden, S. C		1										1		
Carbondale, Pa	17,040	7					2							
Clinton, Mass	13,075	5					1				1	2		
Columbus, Ga	20,544	7												
Columbus, Ind		0							1					
Concord, N. H	21,497	13			2							1		
Cumberland, Md	21,839	8	3		1		1				4		4	
Dunkirk, N. Y		4												
Gloucester, Mass	24,398	12										2		
Harrison, N. J	14,498	3												
Kearny, N. J	18,659	8	1		6		1		2					
La Fayette, Ind	20,081	7			4									
Lebanon, Pa	19,240						1							
Logansport, Ind	19,050	8										1		
Manistee, Mich							7							
Marinette, Wis	14,610	5			2									
Marlboro, Mass	14,579	7	2											
Massillon, Ohio							1							
Medford, Mass	23,150	15			4		1				1			
Melrose, Mass	15,715	4			15	1	1							
Moline, Ill	24,199	6	1									2	1	
Montclair, N. J	21,150	6					2				1		1	
Morristown, N. J	12,507	4										1		
Nanticoke, Pa	18,507	9			9						3			
Newburyport, Mass	19,240	7	1								1			
North Adams, Mass	22,012	1	1		3									
Northampton, Mass	19,431	12					1				3			
Ottumwa, Iowa	22,012	13					1							
Palmer, Mass		7												
Peekskill, N. Y		3	1											
Plainfield, N. J	22,050				38						2			

¹ Epidemic.

MORBIDITY AND MORTALITY—Continued.

Morbidity and mortality table, cities of the United States, for week ended Feb. 24, 1912—
Continued.

Cities.	Population, United States census, 1910.	Total deaths from all causes.	Diphtheria.		Measles.		Scarlet fever.		Small-pox.		Tuberculosis.		Typhoid fever.	
			Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.
*Cities having less than 25,000 inhabitants—*Continued.														
Pottstown, Pa		4			2									
Saratoga Springs, N. Y		3											1	
South Bethlehem, Pa	19,973	6	1		3							1		
Steelton, Pa	14,246	9	3							1		2	1	
Warren, Pa	11,081	3	2		1									
Wilkinsburg, Pa	18,924	7			1		3					3		1	
Woburn, Mass	15,308	7			7									

STATISTICAL REPORTS OF MORBIDITY AND MORTALITY, STATES OF THE UNITED STATES (Untabulated).

CALIFORNIA.—Month of September, 1911. Population, 2,377,549. Total number of deaths from all causes 2,532, including diphtheria 11, measles 4, scarlet fever 3, tuberculosis 373, typhoid fever 45. Cases reported: Diphtheria 60, measles 232, plague 1, scarlet fever 69, smallpox 19, tuberculosis, pulmonary, 217, typhoid fever 120.

MASSACHUSETTS.—Week ended December 2, 1911. Population of reporting towns, 2,593,485. Total number of deaths from all causes 675, including diphtheria 4, measles 2, scarlet fever 3, tuberculosis 62, typhoid fever 5. Cases reported: Diphtheria 136, measles 180, scarlet fever 114, tuberculosis 99, typhoid fever 38.

Week ended December 9, 1911. Total number of deaths from all causes 713, including diphtheria 13, scarlet fever 2, smallpox 2, tuberculosis 64, typhoid fever 6. Cases reported: Diphtheria 142, measles 202, scarlet fever 126, tuberculosis 133, typhoid fever 30.

Week ended December 16, 1911. Total number of deaths from all causes 708, including diphtheria 10, measles 4, tuberculosis 76, typhoid fever 7. Cases reported: Diphtheria 139, measles 309, scarlet fever 154, tuberculosis 146, typhoid fever 30.

Week ended December 23, 1911. Total number of deaths from all causes 670, including diphtheria 10, measles 3, scarlet fever 3, tuberculosis 66, typhoid fever 6. Cases reported: Diphtheria 143, measles 251, scarlet fever 109, smallpox 2, tuberculosis 130, typhoid fever 27.

Week ended December 30, 1911. Total number of deaths from all causes 803, including diphtheria 9, measles 3, scarlet fever 2, tuberculosis 66, typhoid fever 3. Cases reported: Diphtheria 164, measles 251, scarlet fever 110, tuberculosis 116, typhoid fever 22.

NEW JERSEY.—Four weeks ended February 10, 1912. Population, 2,537,167. Total number of deaths from all causes 3,696, including diphtheria 61, measles 14, scarlet fever 16, tuberculosis 424, typhoid fever 28.

FOREIGN AND INSULAR.

AUSTRALIA.

Sydney—Examination of Rats.

The following information was taken from the bulletin issued by the department of health of New South Wales:

During the three weeks ended January 27, 1912, 1,286 rats were examined for plague infection. No plague-infected rat was found.

The last case of human plague was reported May 29, 1909.

The last plague-infected rat was found April 25, 1910.

CHILE.

Sanitary Measures at Chilean Ports.

The following translation of a decree issued December 26, 1911, by the ministry of foreign relations of Chile was received from Minister Fletcher at Santiago de Chile:

1. The only diseases liable to quarantine, for the purposes of sanitary treatment of the ships in ports of the Republic, are bubonic plague, cholera, and yellow fever.

2. In the case of a person suffering from leprosy, trachoma, or other transmissible chronic disease, the ship shall be received in free pratique on condition that the patient, passenger, or member of the crew be strictly and satisfactorily isolated on board the ship, not being permitted to disembark at any time, and his articles of personal use being disinfected.

3. The ships that put into Chilean ports with persons aboard sick with beriberi will not have to submit, merely because of the presence of this disease among their passengers or crew, to any sanitary measures, and receive free pratique.

4. In respect to the sanitary treatment of vessels infected or suspected of infection proceeding from the north, the sanitary doctors of the different ports of the Republic shall attend strictly to the treatment prescribed by the chief of the sanitary station of Africa, in accordance with the provision of decree No. 2909 of June 26, 1909.

EGYPT.

Cairo—Typhus Fever.

Deputy Consul Belrose reports the occurrence of 6 deaths from typhus fever at Cairo during the two weeks ended January 27.

GREECE.

Cerebrospinal Meningitis.

Consul General Gale reports February 15: During the two weeks ended February 13 a death from cerebrospinal meningitis occurred at Piraeus. The disease is prevalent in practically all parts of the country.

HAWAII.

Plague-Infected Rats at Honokaa.

Chief Quarantine Officer Ramus reports the finding of 49 plague-infected rats at Honokaa during the week ended March 2.

Mosquito-eradication Measures at Honolulu.

The following statement of the work of mosquito destruction at Honolulu was received from Passed Asst. Surg. McCoy, who is detailed as sanitary adviser to the governor of the Territory of Hawaii:

Mosquito-eradication measures conducted at Honolulu from Feb. 12 to 17, 1912, both inclusive.

Inspections of—	Total inspections.	Larvæ found in.	Ordered cleaned.	Oiled.	Drained.	Emptied.	Filled.	Ordered repaired.	Screened.	Stocked with mosquito fish.
Gutters, house................	2,494	31	88	3	5	3	......	36	1	......
Gutters, street...............	322	12	8	24	......	......	......	......	10	5
Standing water..............	458	61	5	95	37	......	42	1	......	15
Cesspools...................	1,026	22	7	6	......	......	4	21	......	......
Privy vaults................	1,131	1	17	14	......	......	......	40	......	......
Holes and low places.........	423	56	3	32	15	......	76	6	......	......
Catch basins................	380	5	18	2	......	1	1	1	......	......
Leaky fixtures..............	87	1	......	......	......	......	......	45	......	......
Plants, etc.................	131	......	......	......	......	......	......	......	......	......
Swamps....................	21	6	......	......	2	......	......	......	......	2
Ponds.....................	49	7	......	1	1	......	......	......	......	6
Troughs and tanks...........	224	11	12	......	7	17	......	......	......	5
Tubs or other receptacles.....	643	105	3	1	......	219	......	......	4	......
Tin cans, bottles............	994	94	52	......	......	402	......	......	......	......
Water barrels...............	727	30	......	1	......	346	......	......	1	8
Vacant houses..............	97	3	......	......	......	......	......	......	......	......
Grease traps...............	80	......	......	......	......	......	......	......	......	......

Legal notices to abate nuisances served.. 284
Nuisances abated.. 213
Wagon loads of tin cans, bottles, etc., collected... 43

ITALY.

Naples—Examination of Emigrants.

Surg. Geddings reports:

Vessels inspected at Naples and Palermo week ended February 17, 1912.

NAPLES.

Date.	Name of ship.	Destination.	Steerage passengers inspected and passed.	Pieces of baggage inspected and passed.	Pieces of baggage disinfected.
Feb. 12	San Giovanni..................	New York........	....	....	....
13	Luisiana.....................	....do............	269	15	310
14	Venezia......................	....do............	466	90	580
14	Oceania......................	....do............	747	120	950
15	San Giovanni.................	....do............	233	10	280
17	Cedric.......................	....do............	776	110	890
	Total......................		2,491	345	3,010

PALERMO.

Date.	Name of ship.	Destination.	Steerage passengers inspected and passed.	Pieces of baggage inspected and passed.	Pieces of baggage disinfected.
Feb. 12	Mongibello...................	New York........	....	....	....
12	Luisiana.....................	....do............	....	....	....
16	San Giovanni.................	....do............	107	125	80
17	Franconia....................	....do............	....	....	....
17	Argentina....................	....do............	....	....	....
	Total......................		107	125	80

Consul De Soto has reported smallpox at Palermo as follows: From June 4 to November 25, 1911, 2,428 cases with 1,104 deaths; from November 26, 1911, to February 10, 1912, 2,182 cases with 735 deaths; and during the week ended February 17, 97 cases with 31 deaths.

MEXICO.

Typhus Fever.

At Aguascalientes Consul Schmutz reported 1 death from typhus fever during the week ended February 18.

At Guadalajara Consul Magill reported 1 case with 1 death during the week ended February 24.

At Mexico City Consul General Shanklin reported the occurrence of 53 cases with 11 deaths during the week ended January 20.

Yellow Fever in Merida and Vicinity.

During the week ended February 24, 3 cases of yellow fever were reported at Merida. The total number of cases reported since August 1, 1911, is 63 with 29 deaths.

At the hacienda of Kambul, 57 kilometers distant from Merida, 7 deaths from yellow fever were reported under date of February 27.

RUSSIA.

Typhus Fever.

At Odessa Consul Grout reports the occurrence of 132 cases of typhus fever with 6 deaths during the week ended February 3.

At St. Petersburg Vice Consul Vezey reports 12 cases of typhus fever with 1 death for the 2 weeks ended January 27.

SOUTH AFRICA.

Plague at Durban, Natal—Rat Infection.

Consul Stewart reports January 27: Two cases of plague, both of which were fatal, have developed since the beginning of the outbreak, January 14. Mortality among rats continues and 8 foci of rat infection were reported to January 26. All but one of these foci are along the wharf frontage. One discovered January 23 is situated near the business center of the city.

Plague Rats in Durban—Precautionary Measures.

The following information, dated January 27, was received from the department of the interior of the Union of South Africa:

Infected rats were found at two places within the Durban borough January 21 and 23, respectively, and there are several foci of rat infection in the harbor area. Precautionary measures are being carried out on an extended scale. The premises in which rats have been found have been thoroughly disinfected and stringent precautions have been taken to prevent the escape of rodents from such premises. The greatest care is being exercised to prevent the possibility of infection being conveyed by means of cargo of every description. The systematic destruction of rats is being undertaken and thorough sanitary inspection made.

CHOLERA, YELLOW FEVER, PLAGUE, AND SMALLPOX.

REPORTS RECEIVED DURING WEEK ENDED MAR. 15, 1912.

[These tables include cases and deaths recorded in reports received by the Surgeon General, Public Health and Marine-Hospital Service, from American consuls through the Department of State and from other sources.]

CHOLERA.

Places.	Date.	Cases.	Deaths.	Remarks.
India:				
Bassein	Jan. 14–20	8	6	
Madras	Jan. 29–Feb. 3	19	18	
Negapatam	Jan. 14–20		23	
Indo-China:				
Saigon	Jan. 16–29	291	196	
Turkey in Asia and Europe:				
Provinces	Jan. 6–21	53	50	
Aleppo	Feb. 4–10	10	7	

YELLOW FEVER.

Brazil:				
Ceará	Jan. 1–31		1	
Manaos	Feb. 4–10		5	
Mexico:				
Kambul, hacienda	Feb. 21–27		7	
Merida	....do	3		

PLAGUE.

Brazil:				
Para	Feb. 11–17		2	
Egypt:				
Provinces	Jan. 26–Feb. 15			
Assouan	Jan. 29–Feb. 15	13	7	
Garbieh	Feb. 9–12	3		
Kena	Feb. 15	3	1	
India:				
Bombay	Jan. 29–Feb. 3	16	11	
Karachi	....do	15	15	
Indo-China:				
Saigon	Jan. 16–22	2	2	
Java:				
Pasoeroean Residency	Jan. 21–27	5	4	
Mauritius	Dec. 15–21	5	3	
Peru:				
Trujillo	Feb. 21	34		In the lazaretto.

SMALLPOX.

Canada:				
Fernie	Feb. 26–Mar. 2	2		
Montreal	....do	1		
Ottawa	....do	9		
Quebec	....do	7	1	
China:				
Chienghai	Jan. 29–Feb. 3			Present.
Hankow	Jan. 21–27	1		
Hongkong	....do	40	28	
Kityang	....do			Do.
Shanghai	Jan. 30–Feb. 4	1	2	Deaths among natives.
France:				
Paris	Feb. 4–10	5		
Great Britain:				
West Hartlepool	Feb. 18–24	1		
Hungary:				
Budapest	Feb. 4–10	25		
India:				
Bombay	Jan. 29–Feb. 3	11	6	
Italy:				
Leghorn	Feb. 18–24	1		
Naples	Feb. 11–17	6		
Palermo	....do	97	31	
Turin	Feb. 12–18	2		
Japan:				
Kobe	Jan. 22–28	1		From s. s. Shingo Maru.

CHOLERA, YELLOW FEVER, PLAGUE, AND SMALLPOX—Continued.

Reports Received during Week ended Mar. 15, 1912.

SMALLPOX—Continued.

Places.	Date.	Cases.	Deaths.	Remarks.
Java:				
Batavia	Jan. 21–27	3	1	
Mexico:				
Juarez	Feb. 18–24	3	1	
Magdalena	Feb. 11–24	91	2	
Mazatlan	Feb. 21–27		1	
Mexico	Jan. 14–20	15	8	
Salina Cruz	Feb. 11–17	2	1	
San Luis Potosi	Dec. 24–30		1	
Russia:				
St. Petersburg	Feb. 3	10	2	
South Africa:				
Durban	Jan. 21–27	1		
Johannesburg	Jan. 7–27	29		
Spain:				
Barcelona	Feb. 6–12		1	
Cadiz	Jan. 1–31		8	
Valencia	Feb. 11–17	16		
Teneriffe:				
Santa Cruz	Feb. 4–17		7	
Turkey in Asia:				
Constantinople	Feb. 12–18		12	
Uruguay:				
Montevideo	Dec. 1–31	2		

REPORTS RECEIVED FROM DEC. 30, 1911, TO MAR. 8, 1912.

[For reports received from July 1, 1911, to Dec. 29, 1911, see PUBLIC HEALTH REPORTS for Dec. 29, 1911. In accordance with custom, the tables of epidemic diseases are terminated semiannually and new tables begun.]

CHOLERA.

Places.	Date.	Cases.	Deaths.	Remarks.
Arabia:				
Hodeida	Jan. 21	2	1	
Ras-el-Ketib	Dec. 27–Jan. 1			Total cases, 22; deaths, 12; mainly in the military hospital.
Austria-Hungary:				
Coastland—				
Capodistria	Dec. 14–24	2	2	
Croatia and Slavonia				Total Oct. 22–Dec. 16: Cases, 36.
Sriem	Oct. 22–Dec. 16	36		
Hungary				Total Nov. 19–Dec. 23: Cases, 37.
Backs-Bodog	Dec. 10–16	9	5	
Jasz-Nagykun-Szolnok	Dec. 3–23	11	7	
Torontal	Nov. 19–Dec. 16	17	2	
Bulgaria:				
Burgas	Nov. 22–23	2	2	
Varna	Nov. 6	1		
China:				
Hongkong	Jan. 14–20	1	1	
Dutch East Indies				Total Sept. 24–Dec. 2: Cases, 1,637; deaths, 1,167.
Batavia	Nov. 12–Dec. 23	21	8	Free Dec. 31.
India:				
Bahrein Island	Nov. 27–Dec. 30		260	In the Persian Gulf.
Calcutta	Nov. 5–Jan. 28		378	
Madras	Nov. 26–Jan. 27	433	357	Madras Presidency, Nov. 1–Dec. 31: Cases, 10,436; deaths, 6,545.
Rangoon	Oct. 1–Nov. 30	6	3	
Indo-China:				
Saigon	Nov. 20–Jan. 15	1,121	782	
Italy				Total June 8–Dec. 31: Cases, 15,985; deaths, 6,022.
Provinces—				
Caltanisetta	Nov. 26–Dec. 31	9	7	
Girgenti	do	105	57	
Messina	Nov. 26–Dec. 2	3	2	
Syracuse	Nov. 26–Dec. 23	15	9	
Malta	Nov. 19–Dec. 10	6	6	Dec. 23 declared free from cholera.
Montenegro	Nov. 4–11	9	5	

CHOLERA, YELLOW FEVER, PLAGUE, AND SMALLPOX—Continued.

Reports Received from Dec. 30, 1911, to Mar. 8, 1912.

CHOLERA—Continued.

Places.	Date.	Cases.	Deaths.	Remarks.
Persia:				
Adaban	Nov. 4	1	1	
Kermanshah	Dec. 31			Present.
Philippine Islands:				
Province—				
Union	Oct. 29–Dec. 4	5	5	
Roumania				Total Sept. 9–Dec. 13: Cases, 192; deaths, 42, including report, p. 2094, vol. 1.
Districts—				
Braila	Sept. 11–Dec. 13	84	11	Including cases previously reported.
Convoluri	Oct. 31–Nov. 28	21	1	
Doliju	Nov. 6–Dec. 13	19	4	
Jalonitza	Oct. 31–Nov. 28	4		
Konstanza	Oct. 30–Nov. 28	8		
Prahova	Nov. 6–23	1	1	
Talomita	do	2		
Tulcea	Nov. 24–Dec. 13	15	1	
Servia				Total year 1911: Cases, 95; deaths, 51, including report, p. 2095, vol. 1.
Belgrade, district	Nov. 26–Dec. 16	6	4	Declared free Dec. 31.
Siam:				
Bangkok	Nov. 5–Dec. 30		559	
Straits Settlements:				
Singapore	Nov. 5–18	3	3	
Tripoli:				
Tripoli	Oct. 13–Jan. 24			Cases, 2,000; deaths, from 1,000 to 1,200.
Tunis Regency				Total Nov. 25–Jan. 4: Cases, 462; deaths, 323. No cases since Jan. 10.
Beja district	Nov. 25–Dec. 21	71	20	
Bizerta district	Nov. 25–Dec. 5	9	15	
Turkey in Asia				Provinces in Asia and Europe, Apr. 16–Dec. 30, 1911: Deaths, 6,111, excluding Constantinople. Mainly among troops.
Acre	Jan. 21	7	6	In vicinity.
Adana	Dec. 2–6	16	5	
Aleppo	Jan. 26–Feb. 3	16	8	
Amara	Oct. 15	1	1	
Basra	Oct. 22–28	14	10	
Erzeroum, vilayet	Sept. 11–16	50	28	
Erzeroum	do	11	8	
Kaifa	Dec. 8			Present.
Kerbelah	Oct. 20–28	10	10	
Kharput	Nov. 19–Dec. 30	47	47	
Jiddah	Dec. 2–24	323	310	
Mekka	Dec. 4–24	905	879	Sept. 1–Dec. 24: Cases, 1,648; deaths, 1,565.
Mersina	Dec. 1–7	2	1	
Osmania	Dec. 1–6	2	4	
Sinope	Dec. 7	2	1	
Trebizond and vicinity	Sept. 18–23	64	34	
Tripoli	Jan. 4			Present.
Turkey in Europe:				
Constantinople	Oct. 24–Feb. 3	8	2	
Durazzo	Dec. 7–13	2		
Janina	Jan. 14–22	17	8	
Loros	Jan. 22	12	7	
Saloniki, vilayet	Nov. 6–19	4	3	In Serres.

YELLOW FEVER.

Places.	Date.	Cases.	Deaths.	Remarks.
Brazil:				
Manaos	Nov. 19–Feb. 3		13	
Para	Dec. 9–16	1	1	
Pernambuco	Jan. 1–15		2	
Ecuador:				
Bucay	Nov. 16–30	2		
Duran	Dec. 1–15	3	2	
Guayaquil	Nov. 16–Dec. 15	20	11	
Milagro	do	8	1	

CHOLERA, YELLOW FEVER, PLAGUE, AND SMALLPOX—Continued.

Reports Received from Dec. 30, 1911, to Mar. 8, 1912.

YELLOW FEVER—Continued.

Places.	Date.	Cases.	Deaths.	Remarks.
Mexico:				
Espita	Dec. 31–Jan. 6	1		
Maxcanu	....do	1		
Merida	Nov. 12–Feb. 3	15	9	Total Aug. 1–Feb. 24: Cases, 63; deaths, 29.
Puerto Mexico (Coatzocoalcos).	Feb. 28		1	
Salina Cruz	Feb. 4–7			7 cases in the lazaretto from s. s. Ikalis from Guayaquil.
Temax	Dec. 31–Jan. 6	1		
Portuguese Guinea:				
Bolama	Dec. 19–25	1	1	In an engineer on a vessel.
Venezuela:				
Caracas	Nov. 16–Jan. 15	25	8	
La Guaira	Feb. 27			Present.
Sabana Grande	Dec. 12			Epidemic.
West Indies:				
St. Vincent	Feb. 19	1		
At sea	Dec. 17–23	1	1	On a vessel en route from Manaos to Para.

PLAGUE.

Places.	Date.	Cases.	Deaths.	Remarks.
Algeria:				
Philippeville	Oct. 19–Nov. 11	8	2	Including 5 cases, p. 2096, Vol. XXVI.
Brazil:				
Bahia	Sept. 1–30		2	
Para	Dec. 24–Jan. 27	18	10	
Pernambuco	Oct. 16–Jan. 16		4	
Rio de Janeiro	Nov. 12–Dec. 23	6	3	
British East Africa:				
Kismayu	Oct. 15–25	2		1 case pneumonic.
British South Africa:				
Durban	Jan. 17	2	1	
Chile:				
Iquique	Nov. 12–Jan. 6	10	4	
Pisagua	Nov. 1–30	8		
China:				
Amoy	Jan. 13		1	
Hongkong	Dec. 9–Jan. 13	7	5	
Dutch East Indies:				
Java				Total Mar. 1–Dec. 30: Cases, 1,817; deaths, 1,324.
Pasoeroean Residency, Malang District.	Nov. 12–Jan. 20	72	36	
Soerobaya	Oct. 17–27	2		
German East Africa:				
Dar-es-Salaam	Nov. 13–15	1	1	From the interior via Bergamogo.
Ecuador:				
Guayaquil	Nov. 16–Dec. 15	102	42	
Egypt				Total Jan. 1–Dec. 31, 1911: Cases, 1,656; deaths, 1,041, including cases previously reported.
Provinces—				
Assiout	Jan. 1–25	12	9	Sept. 11–16: Cases, 50; deaths, 28.
Assouan	Jan. 1–Feb. 15	14	8	
Behera	Jan. 1–25	3	2	Sept. 11–16: Cases, 11; deaths, 8.
Fayoum	Jan. 1–26	1	1	
Galioubeh	Jan. 1–Feb. 8	1	1	Oct. 5–Dec. 26: Cases, 1.
Garbieh	Jan. 1–25	1		
Kena	....do	1	1	Nov. 20–Dec. 13: Cases, 3; deaths, 3.
Minieh	Jan. 1–Feb. 1	3	2	Dec. 13: Cases, 1.
Hawaii:				
Honakaa	Feb. 9–25	3	3	
India:				
Bombay	Nov. 19–Jan. 20	80	70	
Calcutta	Nov. 11–Feb. 6		46	
Karachi	Nov. 26–Jan. 20	39	37	Total year 1911: Cases, 3,273; deaths, 3,046.
Rangoon	Oct. 1–Nov. 30	38	39	
Bombay Presidency and Sind.	Oct. 29–Dec. 30	35,557	25,895	
Madras	Jan. 1–6	1	1	
Madras Presidency	Oct. 29–Dec. 30	4,687	3,770	

CHOLERA, YELLOW FEVER, PLAGUE, AND SMALLPOX—Continued.

Reports Received from Dec. 30, 1911, to Mar. 8, 1912.

PLAGUE—Continued.

Places.	Date.	Cases.	Deaths.	Remarks.
India—Continued.				
Bengal	Oct. 29–Dec. 30	3,893	2,827	
United Provinces	.....do	12,270	10,459	
Punjab	.....do	1,229	895	
Burma	.....do	206	187	
Central Provinces	.....do	6,883	5,234	
Coorg	.....do	75	42	
Mysore State	.....do	4,913	3,801	
Hyderabad State	.....do	10,830	10,038	
Central India	.....do	4,242	3,486	
Rajputana and Ajmere Merwara.	.....do	457	362	
North West Province	Oct. 29–Dec. 9	1	1	Total for India, Oct. 29–Dec. 30: Cases, 85,243; deaths, 66,997. Total, year 1911: Cases, 828,535; deaths, 691,849.
Indo-China:				
Saigon	Nov. 13–Jan. 1	21		
Mauritius	Nov. 3–Dec. 7	36	26	
Peru:				
Departments—				
Callao	Oct. 1–21	1		In November 1 case, in January 3 cases with 2 deaths.
Chiclayo	.....do	12	4	
Chosika	.....do	1	1	
Lambayeque	.....do	3		
Libertad	.....do	8		
Lima	.....do	13	6	
Philippine Islands:				
Cebu quarantine station	Dec. 4	1		On s. s. Montrose from Shanghai.
Russian Empire:				
Astrakhan, government	Sept. 21–Jan. 7	201	180	Including 73 cases and 63 deaths reported on page 2098, Vol. I.
Siam:				
Bangkok	Nov. 4–Dec. 2		2	
Straits Settlements:				
Singapore	Nov. 5–Jan. 6	17	16	
Turkey in Asia:				
Jiddah	Jan. 13–15	2		

SMALLPOX.

Places.	Date.	Cases.	Deaths.	Remarks.
Algeria:				
Algiers	Nov. 1–30		1	
Oran	Jan. 1–31	2	1	
Arabia:				
Aden	Nov. 28–Jan. 15	5	3	And vicinity.
Argentina:				
Buenos Aires	Oct. 1–31		6	
Rosario	Oct. 1–Nov. 30		31	
Austria-Hungary:				
Bohemia	Jan. 14–20	1		
Galicia	Dec. 24–30	1		
Krain	Jan. 14–20	7		
Trieste	Dec. 3–9	1		From s. s. Baron Call from Beirut.
Tyrol	Jan. 14–20	1		
Brazil:				
Bahia	July 1–31		1	
Pernambuco	Oct. 16–Jan. 15		388	Report for Oct. 1–15 not received.
Rio de Janeiro	Nov. 26–Jan. 20	4	1	
Santos	Dec. 12–23		1	
Canada:				
British Columbia—				
Nelson	Dec. 24–30	1		
Victoria	Feb. 4–10	1		
Manitoba—				
Winnipeg	Jan. 14–20	1		
Ontario—				
Kingston	Dec. 19–23	1		
Ottawa	Dec. 10–Feb. 17	60		
Sarnia	Oct. 17–Dec. 31	42		
Toronto	Jan. 6–Feb. 10	2	1	
Windsor	Feb. 4–10	2		
Quebec—				
Montreal	Dec. 17–Feb. 17	20		
Quebec	Dec. 10–Feb. 24	235	1	

CHOLERA, YELLOW FEVER, PLAGUE, AND SMALLPOX—Continued.

Reports Received from Dec. 30, 1911, to Mar. 8, 1912.

SMALLPOX—Continued.

Places.	Date.	Cases.	Deaths.	Remarks.
Ceylon:				
Colombo	Nov. 12–18	1		
Chile:				
Iquique	Dec. 10–16	2		
La Serena	Nov. 21–30	14		
Santiago	Nov. 1–30	685	343	
Talcahuano	Nov. 26–Dec. 23	14	3	
Valparaiso	Dec. 3–9	43		Feb. 10—Present.
China:				
Canton	Nov. 11–Dec. 30	40	6	
Chungking	Nov. 18–Jan. 6			Present.
Hongkong	Nov. 12–Jan. 20	169	126	
Nanking	Dec. 10–Feb. 3			Do.
Shanghai	Dec. 11–Jan. 21		3	
Cuba:				
Habana	Dec. 19–Jan. 19	2		Case Dec. 19 from German s. s. Frankenwald, from Spain and Canary Islands; case Jan. 19 from s. s. Mexico.
Egypt:				
Cairo	Dec. 10–Jan. 14	3		
France:				
Marseille	Jan. 1–31		3	Nov. 1–30, 1 death.
Paris	Dec. 3–Jan. 27	63	5	
Germany				Total, Dec. 31–Feb. 17: Cases, 23.
Hamburg	Jan. 21–27	1		
Great Britain:				
Bristol	Jan. 29–Feb. 3	2		
London	Jan. 14–Feb. 10	4	1	
India:				
Bombay	Nov. 19–Jan. 27	137	66	
Calcutta	Nov. 19–Feb. 6		18	
Madras	Nov. 26–Jan. 27	56	33	
Rangoon	Oct. 1–Nov. 30	29	9	
Indo-China:				
Saigon	Nov. 13–Jan. 29	25	1	
Italy:				
Genoa	Dec. 1–Jan. 31	33	2	
Leghorn	Dec. 16–Feb. 17	91	1	
Messina	Nov. 19–Jan. 31		6	
Naples	Dec. 3–Feb. 10	62	1	
Palermo	Nov. 26–Feb. 10	2,182	735	
Turin	Jan. 15–Feb. 4	3		
Japan:				
Arima-Mura	Nov. 12–18	6	1	11 miles east from Kobe.
Kanagawa, ken	Dec. 17–23	1		
Kobe	Jan. 22–28	1	1	Jan. 20, 1 case from s. s. Suveric from Hongkong.
Yokohama	Jan. 22	1		From s. s. Hydra from New York via Suez.
Java:				
Batavia	Nov. 12–Jan. 20	24	5	
Malta	Dec. 24–Jan. 6	2	1	
Mexico:				
Aguascalientes	Dec. 18–Feb. 11		5	
Chihuahua	Nov. 20–Feb. 11	92	36	
Coahuila, State	Oct. 1–30		16	
Guadalajara	Jan. 14–Feb. 17	4	2	
Juarez	Dec. 19–Feb. 10	9	4	
Magdalena	Dec. 23–Feb. 7		47	Feb. 7, 62 cases present.
Manzanillo	Feb. 18–24	1		
Mazatlan	Dec. 11–Jan. 30		9	Feb. 13, 33 cases in the lazaretto.
Mexico	Nov. 26–Jan. 13	46	25	
Monterey	Dec. 11–24		2	
Porfirio Diaz	Dec. 3–Feb. 24		32	
San Antonio	Jan. 1–21	12	9	
San Carlos	do			Present.
Sandoval	Dec. 16			Do.
San Ignacio	Jan. 8	3		
Saric	Jan. 21–27		6	
Santa Ana	Jan. 8	4		
San Luis Potosi	Nov. 12–Dec. 2	3		
Tampico	Dec. 1–Feb. 20		10	
Tapachula	Nov. 1–Dec. 31		14	
Portugal:				
Lisbon	Dec. 9–Feb. 17	35		
Russia:				
Batum	Dec. 1–31	1		
Libau	Dec. 17–23	1		

CHOLERA, YELLOW FEVER, PLAGUE, AND SMALLPOX—Continued.

Reports Received from Dec. 30, 1911, to Mar. 8, 1912.

SMALLPOX—Continued.

Places.	Date.	Cases.	Deaths.	Remarks.
Russia—Continued.				
Moscow	Nov. 19–Feb. 3....	26	10	
Odessa	Nov. 26–Jan. 13....	10	1	
Reval	Nov. 1–30	1		
Riga	Dec. 24–Jan. 27....	16		Oct. 1–Nov. 30; deaths, 2.
St. Petersburg	Nov. 19–Jan. 27...	112	20	
Warsaw	Nov. 5–Dec. 2....		185	
Siam:				
Bangkok	Nov. 5–Dec. 30....		626	
Siberia:				
Omsk	Jan. 1–31	7		
Spain:				
Cadiz	Nov. 1–Dec. 31....		14	
Madrid	Dec. 1–Jan. 31....		3	
Malaga	Nov. 1–30		45	
Seville	Dec. 1–31		5	
Valencia	Dec. 3–Feb. 10....	149	11	
Straits Settlements:				
Singapore	Nov. 19–Jan. 13...	18	7	
Switzerland:				
Cantons—				
Oberwalden	Jan. 14–20	1		
Zurich	Dec. 3–23	6		
Teneriffe:				
Santa Cruz	Dec. 3–Feb. 3....		35	
Turkey in Asia:				
Beirut	Dec. 3–Feb. 17....	855	77	
Turkey in Europe:				
Constantinople	Dec. 4–Feb. 4....		53	
Uruguay:				
Montevideo	Sept. 1–Nov. 30....	23	4	
Venezuela:				
Caracas	Nov. 1–Jan. 15....	11	2	
Zanzibar:				
Zanzibar	Oct. 28–Dec. 15....	3	2	

MORTALITY.

WEEKLY MORTALITY TABLE, FOREIGN AND INSULAR CITIES.

Cities.	Week ended—	Estimated population	Total deaths from all causes.	Deaths from—										
				Tuberculosis.	Plague.	Cholera.	Yellow fever.	Smallpox.	Typhus fever.	Typhoid fever.	Scarlet fever.	Diphtheria.	Measles.	Whooping cough.
Aleppo	Feb. 10	200,000						7						
Aix-la-Chapelle	Feb. 3	157,509	64	8								2		
Amsterdam	Feb. 17	580,962	188	25						1		3		6
Antwerp	Feb. 10	316,604	85	5							2	1		1
Athens	Feb.1–13	250,010	172	33							1	1		
Barcelona	Feb. 24	591,272		63					1		7	1	6	1	
Beirut	Feb. 10	80,000							10		8				
Belfast	Feb. 17	385,492	248	29							2				17
Berlin	Feb. 10	2,087,929	660	105								6	28	4	2
Birmingham	Feb. 17	842,512	285								2	2		1	16
Bombay	Feb. 3	977,822	700	40	11				27					5	
Bordeaux	Feb. 17	253,000	198	18								2	2		
Do	Feb. 24	253,000	120	12									2		1
Bremen	Feb. 3	246,850	70	7									4		
Bristol	Feb. 17	359,400	172	10								1	1		
Brussels	Feb. 10	739,684	257	25							2	1	1	4	1
Do	Feb. 17	739,684	242	31									1	1	2
Budapest	Feb. 3	1,000,000										5	2	6	1
Catania	Feb. 23	207,000	101	5								2	1	2	

MORTALITY—Continued.

Weekly mortality table, foreign and insular cities—Continued.

Cities.	Week ended—	Estimated population.	Total deaths from all causes.	Tuberculosis.	Plague.	Cholera.	Yellow fever.	Smallpox.	Typhus fever.	Typhoid fever.	Scarlet fever.	Diphtheria.	Measles.	Whooping cough.
Christiania	Feb. 10	245,000	64	11							2	1		
Do	Feb. 17	245,000	81	14								1	1	
Coburg	Feb. 3	24,299	7	1										
Cologne	Feb. 10	525,671	153	20								1	4	2
Copenhagen	Feb. 3	465,000	123	19								2		2	3
Dalny	do	47,695	22	2							1			
Dresden	do	555,800	159	19						1	2	5		4
Dublin	Feb. 10	406,536	219	28						1	1	3	4	5
Dundee	Feb. 17	171,006	61	3								2		
Durban	Jan. 27	69,165	22	1						1				
Edinburgh	Feb. 17	321,200	139	14									4	1
Erfurt	Feb. 10	126,010	47	4								3		
Frankfort	Jan. 27	423,600	81								1	2		
Do	Feb. 3		98									3		
Glasgow	Feb. 23	785,600	387							2	1	3	42	3
Gothenberg	Feb. 10	170,100	55	10						1			1	
Greenock	Feb. 17	75,900	39										4	4
Guadalajara	Feb. 24	119,468	67						1					
Hamburg	Feb. 10	953,079	304	42						1	5	7		1
Do	Feb. 17		263	30							3	12	2	2
Havre	do	136,159	76	11									1	1
Hongkong	Jan. 27	336,488	76					28						
Juarez	Feb. 24	6,500	8	1				1						
Karachi	Feb. 3	148,000	111		15								12	
Kharput	do	21,000										2		
Konigsberg	do	251,000	108	11									2	7
Do	Feb. 10		121	8									4	4
Leeds	Feb. 17	445,568	6							1	2	2		1
Leghorn	Feb. 24	104,000	35											
Leipzig	Feb. 3	605,755	175	21						2		3		1
Liege	do	167,521	56	3							1			1
Liverpool	Feb. 17	752,055	288	24							3	2	2	7
Lubeck	do	100,000	34	4									1	
Lyon	Jan. 13 / Feb. 10	523,796	935	147						4	1	12	21	
Madras	Feb. 3	518,660	459			18		6		1				
Manaos	Feb. 10	52,000	48	4			5							
Manchester	Feb. 17	631,533	342	21								1	11	16
Mannheim	Jan. 27	193,500	38	4						1				
Do	Feb. 3	200,000	55	8						1				
Mazatlan	Feb. 27	22,000	19	3				1						
Mexico	Jan. 20	719,052	414					8	11			1	5	4
Montreal	Mar. 2	466,197	159	23						2		1	3	
Moscow	Feb. 8	1,500,000	798	105					1	4	19	11	29	10
Munich	Feb. 10	610,000	198	23									1	
Nagasaki	Feb. 4	179,257	37	8						1				
Nantes	Feb. 18	170,455	72	15						1		2		
Do	Feb. 25		68	20							1			
Niagara	do	10,000	4									1		1
Nottingham	Feb. 10	259,942	130	14								3	4	2
Ottawa	Feb. 24	90,000	26	5										
Palermo	Feb. 17	340,000	155	3				31		4	1			
Para	do	185,000	90	3			2							
Paris	Feb. 10	2,888,110	1,210	255						7	3	7	21	9
Porfirio Diaz	Feb. 24	16,000	7	1				1						
Port of Spain	Jan. 27 / Feb. 17	60,000	122	23						12				
Prague	Feb. 3	225,204	78	19							1		3	1
Do	Feb. 10		98	12							1		5	
Progresso	Feb. 17	6,959	7	1									1	
Quebec	Mar. 2	78,200								1				1
Rome	Nov. 4 / Nov. 18	523,796	49								16	4		2
Saigon	Jan. 22	220,000	124		2	122								
Do	Jan. 29		75			74		1						
St. Petersburg	Feb. 3	1,907,708	116					2		19	13	36	13	7
Salina Cruz	Feb. 17	6,138	9					1		1				
Santa Cruz	Feb. 10	46,000	14	3				2						
Santiago de Cuba	Feb. 24	53,614	16	1								1		
Shanghai	Feb. 4	500,000	212	26				2				3	3	22

MORTALITY—Continued.

Weekly mortality table, foreign and insular cities—Continued.

Cities.	Week ended—	Estimated population.	Total deaths from all causes.	Deaths from—										
				Tuberculosis.	Plague.	Cholera.	Yellow fever.	Smallpox.	Typhus fever.	Typhoid fever.	Scarlet fever.	Diphtheria.	Measles.	Whooping cough.
Singapore	Jan. 20	303,328	22	2						2				
Smyrna	Jan. 27	400,000	98	12						2	1			
Southampton	Feb. 17	120,891	39	3								1		
Do.	Feb. 24		38	4								1		3
Stettin	Feb. 10	237,000	87	5								1		
Stockholm	Feb. 3	343,832	113	12							4	2		
Do.	Feb. 10		115	18							2	4		
Stoke-on-Trent	Feb. 17	237,153	84	7						2	2		3	
Talcahuano	Feb. 10	28,000	5	2										1
Tarragona	Feb. 17	23,150	8										1	
Trieste	Feb. 3	233,599	119							2		1	5	1
Do.	Feb. 10		116							1		1	1	4
Turin	Feb. 18	430,770	161	12						1		2	2	
Vienna	Feb. 3	2,064,583	729	116							5	7	5	6
Vigo	Feb. 10	41,500	17	2								2		
Do.	Feb. 17		2									1		
Winnipeg	Feb. 24	151,958	24								1			

MORTALITY — FOREIGN AND INSULAR — COUNTRIES AND CITIES (Untabulated).

ALGERIA—*Algiers.*—Month of January, 1912. Population 172,397. Total number of deaths from all causes 344, including diphtheria 1, measles 2, tuberculosis 45, typhoid fever 9.

Oran.—Month of January, 1912. Population 123,086. Total number of deaths from all causes 261, including measles 1, smallpox 1, tuberculosis 22, typhoid fever 4.

BRAZIL—*Pernambuco.*—Two weeks ended January 15, 1912. Population 225,000. Total number of deaths from all causes 397, including plague 2, smallpox 65, tuberculosis 57, yellow fever 2.

FRANCE—*Marseille.*—Month of January, 1912. Population 550,619. Total number of deaths from all causes 861, including diphtheria 12, measles 1, scarlet fever 2, smallpox 3, tuberculosis 115, typhoid fever 12.

GREAT BRITAIN.—Week ended February 10, 1912:

England and Wales.—The deaths registered in 77 great towns correspond to an annual rate of 21.6 per 1,000 of the population, which is estimated at 17,559,219.

Ireland.—The deaths registered in 21 principal town districts correspond to an annual rate of 31 per 1,000 of the population, which is estimated at 1,157,014. The lowest rate was recorded at Queenstown, viz, 6.6, and the highest at Belfast, viz, 41.7 per 1,000.

Scotland.—The deaths registered in 18 principal towns correspond to an annual rate of 22.8 per 1,000 of the population, which is esti-

mated at 2,182,400. The lowest rate was recorded at Govan, viz, 10.4, and the highest at Perth, viz, 31.8 per 1,000. The total number of deaths from all causes was 953, including diphtheria 13, measles 62, scarlet fever 3, typhoid fever 3.

ITALY—*Genoa.*—Two weeks ended February 15, 1912. Population, 272,077. Total number of deaths from all causes 123, including diphtheria 2, tuberculosis 15.

Milan.—Month of January, 1912. Population, 602,236. Total number of deaths from all causes 122, including diphtheria 8, scarlet fever 2, tuberculosis 103, typhoid fever 6.

SOUTH AFRICA—*Johannesburg.*—Month of January, 1912. Population, 237,220. Total number of deaths from all causes 396, including diphtheria 3, measles 2, tuberculosis 60, typhoid fever 17.

SPAIN—*Cadiz.*—Month of January, 1912. Population, 67,306. Total number of deaths from all causes 214, including diphtheria 4, smallpox 8, tuberculosis 17, typhoid fever 1.

By authority of the Secretary of the Treasury:

RUPERT BLUE,
Surgeon General,
United States Public Health and Marine-Hospital Service.

34