Report on the etiology and prevention of yellow fever / by George M. Sternberg.

UNITED STATES MARINE HOSPITAL SERVICE

REPORT

ON THE

ETIOLOGY AND PREVENTION

OF

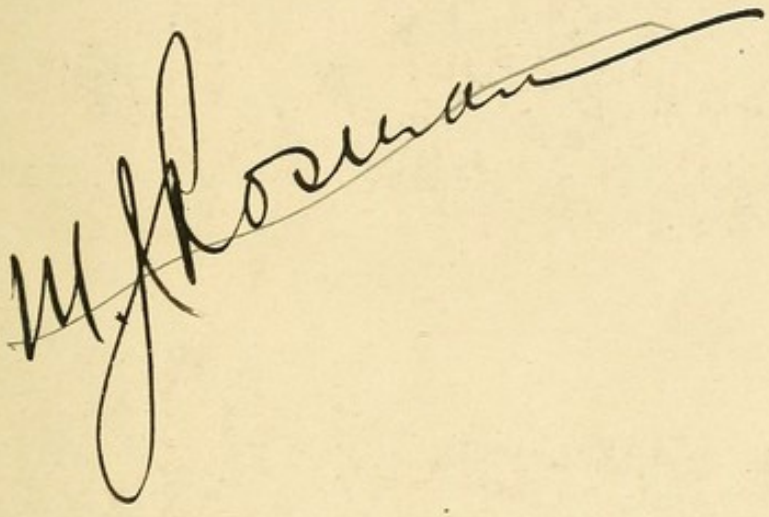
YELLOW FEVER

BY

GEORGE M. STERNBERG

LIEUT. COL. AND SURGEON, U. S. ARMY

Public health bulletin, no. 2



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GEORGE M. STERNBERG,

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Published by order of the Secretary of the Treasury, in accordance with the act of Congress approved March 3, 1887.

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TREASURY DEPARTMENT,

Document No. 1328.

Marine-Hospital Service.

LETTER OF THE SUPERVISING SURGEON-GENERAL.

TREASURY DEPARTMENT,
OFFICE OF THE SUPERVISING SURGEON-GENERAL,
U. S. MARINE HOSPITAL SERVICE,
July 11, 1890.

SIR: Referring to the accompanying report of Maj. George M. Sternberg, surgeon U. S. Army, of his researches relating to the etiology of yellow fever, I have the honor to recommend that it be immediately printed as a special report from this Bureau.

Very respectfully, your obedient servant,

JOHN B. HAMILTON,
Supervising Surgeon-General Marine Hospital Service.

Hon. WILLIAM WINDOM,
Secretary of the Treasury.

Approved, July 11, 1890.

WILLIAM WINDOM,
Secretary of the Treasury.

RESEARCHES

RELATING TO THE

ETIOLOGY AND PREVENTION OF YELLOW FEVER,

MADE BY

GEORGE M. STERNBERG, M. D.,
LIEUT. COLONEL AND SURGEON, U. S. ARMY,

By direction of the President, in pursuance of an act of Congress approved March 3, 1887, "making appropriations for sundry civil expenses of the Government."

SUBMITTED JUNE 21, 1890.

ORDERS.

Special Orders No. 93.

HEADQUARTERS OF THE ARMY,
ADJUTANT-GENERAL'S OFFICE,
Washington, April 23, 1888.

[Extract.]

1. The following order, received from the War Department, is substituted for paragraph 13, Special Orders No. 89, April 18, 1888, from this office, which is, by direction of the Secretary of War, revoked:

WAR DEPARTMENT, WASHINGTON, *April* 21, 1888.

By direction of the President, in pursuance of the authority contained in the provisions of the act of Congress, approved March 3, 1887, "making appropriations for sundry civil expenses of the Government," etc., relating to the methods of preventing the spread of epidemic diseases, Maj. George M. Sternberg, Surgeon U. S. Army, will proceed to the Island of Cuba for the purpose named in the letter of the President addressed to the Secretary of War on the 17th instant, and upon the completion of this duty will return to his proper station and submit his report to the President on or before June 25, 1888.

The travel enjoined is necessary for the public service.

WM. C. ENDICOTT,
Secretary of War.

* * * * * * *

By command of Lieutenant-General Sheridan:

R. C. DRUM,
Adjutant-General.

Special Orders, No. 224.

HEADQUARTERS OF THE ARMY,
ADJUTANT-GENERAL'S OFFICE,
Washington, September 26, 1888.

[Extract.]

* * * * * * *

8. The following order has been received from the War Department:

WAR DEPARTMENT, *Washington, September* 26, 1888.

By direction of the President, Maj. George M. Sternberg, Surgeon U. S. Army, will proceed to Decatur, Ala., and to such other points in the infected districts of the Southern States as he may deem necessary to continue his scientific investigations of yellow fever.

R. MACFEELY,
Acting Secretary of War.

Upon the completion of the duty assigned him in this order, Major Sternberg will return to his proper station.

The travel enjoined is necessary for the public service.

* * * * * * *

By command of Major-General Schofield:

R. C. DRUM,
Adjutant-General.

Special Orders, No. 30.

HEADQUARTERS OF THE ARMY,
ADJUTANT GENERAL'S OFFICE,
Washington, February 5, 1889.

[Extract.]

* * * * * * *

16. The following order has been received from the War Department:

WAR DEPARTMENT, *Washington, February* 4, 1889.

By direction of the President, in pursuance of the authority contained in the provisions of the act of Congress, approved March 3, 1887, "making appropriations for sundry civil expenses of the Government," etc., relating to the methods of preventing the spread of epidemic diseases, Maj. George M. Sternberg, Surgeon U. S. Army, will proceed to the Island of Cuba for the purpose named in the letter of the President addressed to the Secretary of War April 17, 1888, and upon the completion of this duty will return to his proper station and submit his report to the President.

The travel enjoined is necessary for the public service.

WM. C. ENDICOTT,
Secretary of War.

* * * * * * *

By command of Major-General Schofield.

R. C. DRUM,
Adjutant-General.

ACKNOWLEDGMENTS.

I am indebted to the president and trustees of the Johns Hopkins University, of Baltimore, for the title and privileges of Fellow by Courtesy in the University, and to Dr. N. H. Martin, professor of biology, and Dr. Wm. H. Welch, professor of pathology, for laboratory facilities and valuable assistance in various ways.

In Havana, through the courtesy of the governor-general of the island; of Dr. Antonio Pardinas, medical inspector-general; and of Dr. Fernandes Malo, director of the military hospital, I have had free access to the wards of this hospital, and have been able to make numerous autopsies in typical cases of yellow fever. I desire to express my obligations to these gentlemen and to the medical officers of the Spanish army on duty at this hospital. Also to Dr. Emiliano Nunez, director of the new and admirable civil hospital, *Nuestra Senora de las Mercedes.*

Also to Dr. Santos Fernandos, and the gentlemen connected with the bacteriological laboratory of the "Cronica Medica-Quirurgica," for valuable assistance in the preparation of culture material, etc.; to Dr. Daniel M. Burgess, United States sanitary inspector, Marine Hospital Service, at Havana, for assistance at autopsies, and in many other ways during my entire stay in Cuba; to Dr. Carlos Finlay, and his associate in bacteriological work, Dr. Claudio Delgado; to Dr. Francis I. Vildosola for the use of his laboratory, in which my culture material was prepared during the summer of 1889, by Dr. Emilio Martinez. The last-named gentleman has rendered most valuable services as my assistant during the past year both in Cuba and in Baltimore. During my stay at Decatur, Ala., in 1888, I received valuable assistance from Dr. Jerome Cochran, health officer of the State of Alabama, and from Dr. B. F. Cross, Dr. E. J. Conyngton, and Dr. E. M. Littlejohn, practicing physicians in Decatur.

Some of my photomicrographs have been made at the Hoagland Laboratory, Brooklyn, with the kind assistance of Dr. C. N. Hoagland. Dr. James E. Reeves, of Chattanooga, Tenn., has kindly prepared for me a series of sections from twenty-five of my cases, which I have placed beside my own and those of my laboratory assistant, Dr. Mar-

tinez, in the series submitted with this report. Dr. William T. Councilman, associate in pathology in the Johns Hopkins University, has at my request examined and reported upon this entire series of slides.

Dr. F. P. Mall, formerly associate in pathology in the Johns Hopkins University, has also examined for me some pathological material and a series of slides. My thanks are due to the Supervising Surgeon-General of the Marine Hospital Service for promptly filling my requisitions and approving my accounts.

INTRODUCTION.

The present report embodies an account of the researches made by the writer in compliance with the foregoing orders.

My investigations in Brazil and Mexico, made in 1887, were in compliance with orders received about the 1st of May, 1887, in which I am especially instructed to complete my investigations by the 1st of October of the same year. The following is a copy of this order:

WASHINGTON, *April* 29, 1887.

SIR: Referring to the act providing for sundry civil expenses of the Government for the year ending June 30, 1888, especially to the clause providing for the investigation of yellow fever by inoculation, as follows: "And the President is further authorized to use of the same unexpended balance a sum not exceeding $10,000 for the purpose of investigating the merits of the method practiced in Mexico and Brazil for preventing yellow fever by inoculation," you are hereby directed, under authority of said act, to proceed to Rio de Janeiro, where you will collate the documentary and other evidence of the experiments by Dr. Freire. Having thoroughly familiarized yourself with the claims of Dr. Freire, you will proceed in person to inquire at the Jura-Juba Hospital and such other places as may occur to you after your arrival at Rio:

First. The source from which the culture supply is secured, which will involve—

(*a*) The examination of the alleged germ as shown you by those engaged in the business of inoculation.

(*b*) Verification of the cultivation and process of attenuation adopted.

Second. The method of the inoculation, which you will see verified, if practicable, on actual cases.

Third. You will report your opinion on the results attained by the process after a careful examination of the cases which have previously been subjected to inoculation. In forming your judgment of these results you will take into consideration the following points:

(*a*) Personal characteristics of the patient; age, race, nativity, sex, previous susceptibility.

(*b*) The period since last inoculation; number of times exposed to the contagion.

Having completed this study, you will then proceed to Mexico by the shortest and most practicable route and investigate, in the same manner, the method of inoculation practiced by Dr. Carmona y Valle, and the same method will be observed in conducting the investigation.

While your attention is directed specifically to these points and details, with the expectation that they will be carefully kept in view and adopted for your guidance, they are not intended to exclude such additional methods and means of investigation as your judgment may approve in the thorough and careful accomplishment of the purposes of your mission.

In order that every facility may be afforded you for the prosecution of the work, you will make known your errand to the United States minister at Rio de Janeiro

and the United States minister at the City of Mexico, respectively, and request them to use their influence in procuring such access to the hospitals and such other sources of information as you may desire. You will refrain from making publication of your investigations and the conclusions reached by you until you shall have submitted to me the completed report. You will forward your vouchers for traveling expenses, from time to time, to the Supervising Surgeon-General of the Marine-Hospital Service for payment, certifying the same before any consular officer where you may be at the time.

You will also make requisitions on the Supervising Surgeon-General for such scientific appliances as may be necessary to accomplish the object of your journey.

It is expected that your investigation will be completed by the 1st of October.

GROVER CLEVELAND.

Maj. GEORGE M. STERNBERG,
Surgeon, U. S. Army.

In compliance with these orders I submitted a report to the President in March, 1888, which is published in the annual volume of the Marine-Hospital Service for 1889.

In transmitting this report I say:

I have the honor to transmit herewith a detailed report of the investigations which I have made in compliance with your instructions dated April 29, 1887.

The conclusions reached (MS., pp. 280 to 283) are definite so far as the methods of inoculation practiced in Brazil and in Mexico are concerned, but unfortunately the question of the etiology of yellow fever is left in an unsettled state. The limit as to time fixed by my orders, and the fact that the disease was not prevalent either in Brazil or in Mexico at the time of my visits to those countries, have made it impossible for me to make certain researches which I consider extremely important in connection with the subject under investigation.

The orders given me in April, 1888, were intended to afford me the opportunity I desired for continuing my investigations during the epidemic season at one of the permanent centers of infection, but the time was again limited through a misapprehension with reference to the availability of the appropriation after the end of the fiscal year, and I was required to return to my proper station and submit my report to the President "on or before June 25, 1888."

The report submitted in compliance with this requirement was a brief one. At my request it was subsequently returned to me and is embodied in the present report. In the autumn of 1888, yellow fever having appeared in epidemic form in Florida and Alabama, I requested authority to proceed to the infected district for the purpose of continuing my investigations, and received the orders dated September 26, 1888.

I selected Decatur as the locality for my researches, rather than Jacksonville, because the comparatively small mortality in the last-named city led some physicians to question the diagnosis, and because my friend, Dr. Jerome Cochran, State health officer, was at Decatur, and I was confident that he would do all that was in his power to aid me in my scientific researches, an expectation which was fully realized.

Not having arrived at any definite conclusion as to the *specific* cause of the disease under investigation, I again asked to be sent to Cuba during the epidemic season of 1889, and received the orders dated Feb-

ruary 5, 1889, which did not restrict me as to time and enabled me to spend the entire summer in Havana.

The material obtained from thirty autopsies made in Havana during the past summer and the study of the various microörganisms isolated in my culture experiments have fully occupied my time since my return from Cuba.

I have now commenced writing a report because I feel that an account of what I have been doing during the past two years is due, and not because I have brought my investigation to a successful termination, or because I feel that there is nothing more to be done.

No one can regret more than I do that the question of the etiology of yellow fever is not yet solved in a definite manner, but I at least have not to reproach myself with want of diligence or failure to embrace every opportunity for pursuing the research. The difficulties have proved to be much greater than I anticipated at the outset. If the task before me had been to find an organism in the blood, like that of relapsing fever or of anthrax, or an organism in the organs principally involved, as in typhoid fever, or leprosy, or glanders, or in the intestine, as in cholera, the researches I have made could scarcely have failed to be crowned with complete success. But this has not proved to be the case, and among the microörganisms encountered there is not one which by its constant presence and special pathogenic power can be shown indisputably to be the specific infectious agent in this disease.

If I have not succeeded in making a positive demonstration which will satisfy the exactions of science I have at least been able to exclude in a definite manner a majority of the microörganisms which I have encountered in my culture experiments, as well as those which various other investigators (Freire, Carmona, Finlay, Gibier) have supposed to be the specific cause of the disease. I shall endeavor to give an exact account of the characters of these various microörganisms and of the evidence upon which I feel justified in excluding them from consideration from an etiological point of view.

While this is much less than I had aimed at and hoped for, I trust that it will be considered ample compensation for the time and money expended, and I am sure that the information obtained can not fail to be of value in guiding future investigators in this field of research.

I desire here to quote the following paragraphs from a paper read at the quarantine conference held in Montgomery, Ala., in March, 1889, just before my last visit to Havana:

> I may say before going any further that my faith in a living infectious agent as the specific cause of this disease is by no means diminished by my failure thus far to demonstrate the exact form and nature of this hypothetical "germ." The present state of knowledge with reference to the etiology of infectious diseases in general and well known facts relating to the origin and spread of yellow-fever epidemics fully justify such a belief. The *a priori* grounds for such faith I stated as long ago as 1873 in a paper published in the American Journal of the Medical Sciences (July, 1873); and the progress of knowledge since that date has all been in the direction of

supporting this *a priori* reasoning. But yellow fever is by no means the only infectious disease in which satisfactory evidence of the existence of a living infectious agent is still wanting. In the eruptive fevers generally no demonstration has been made of the specific etiological agent—at least none which has been accepted by competent pathologists and bacteriologists. Again, in the infectious disease of cattle known as pleuropneumonia, notwithstanding very extended researches by competent investigators in various parts of the world, no satisfactory demonstration of the germ has been made. The same is true of hydrophobia, in which disease we are able to say with confidence the infectious agent is present in the brain and spinal cord of animals which succumb to rabies; this infectious agent is destroyed by a temperature which is fatal to known pathogenic microörganisms (65° C.), and by various germicide agents, yet all efforts to cultivate it or to demonstrate its presence in the infectious material by staining processes and microscopical examination have thus far been unsuccessful.

You are aware that my first effort to solve the etiology of yellow fever was made 10 years ago. As a member of the Havana yellow-fever commission of the National Board of Health I had an opportunity to make researches which, in advance of the effort, I fondly hoped might lead to a demonstration alike creditable to American science and useful as a basis for preventive and curative measures in this pestilential malady, which has destroyed the lives of so many of our fellow-citizens, and has so largely interfered with the material progress of certain sections of the United States. I knew from personal experience the malignant nature of the disease, and the futility of the various modes of treatment which had been resorted to in the effort to combat it. It was therefore with the deepest interest as well as with strong hopes of success that I went to an endemic focus of the disease to search for the yellow-fever germ. The recent (1873) demonstration of the spirillum of relapsing fever in the blood of patients suffering from this disease, and the recognized facts relating to the etiology of anthrax, considered in connection with the current notions relating to the pathology of yellow fever, led me to hope that the discovery would be an easy one. I was familiar with the most approved methods of mounting and staining microorganisms, and was provided with the best high-power objectives that could be procured, the one-twelfth and one-eighteenth inch homogeneous oil-immersion objectives of Karl Zeiss, of Jena, Germany. Not only did I feel that I was equipped for the recognition of any microörganism which might prove to be present in the blood, but I was prepared to photograph it, and thus to show to others what I might see in blood drawn from the circulation of yellow-fever patients. You know the result of this investigation; ninety-eight specimens from forty-one undoubted cases of yellow fever were carefully studied, and one hundred and five photographic negatives were made, which showed satisfactorily everything demonstrable by the microscope. But no microörganism was discovered. I shall presently show you upon the screen a photomicrograph of yellow-fever blood, made in Havana at the time mentioned, so that you may judge of the performance of my Zeiss one-eighteenth inch objective, and have ocular evidence that no microörganism demonstrable by this magnificent lens was present in it. I may say here that my culture experiments made in Havana last spring, in which blood taken from one of the cavities of the heart as soon as possible after death was introduced into various nutritive media, gave a like negative result.

Out of ten cases in which I made the autopsy, in the military hospital at Havana, a development of microörganisms occurred in two only. In the exceptional cases I obtained a bacillus which subsequent researches showed to be identical with a bacillus constantly found in the alimentary canal of healthy persons—bacterium coli commune of Escherich.

The absence of microörganisms from blood drawn from the finger during life or from the heart after death can not, however, be accepted as evidence that there are no parasitic organisms anywhere in the tissues. The bacillus of typhoid fever, for example, is rarely found in the circulating fluid, although it must be transported in

the blood current to the various organs in which foci of growth are found which contain numerous bacilli. Such foci are especially abundant in the spleen, but even in this organ many thin sections may be made before a single focus of development is encountered.

Having failed to find the yellow fever germ in the blood, we may still admit that, as in typhoid, it is perhaps only to be found in the organs principally involved in the morbid process. This reasoning has led me to give special attention to an examination of the liver and kidney, both by the culture method and by the examination of thin sections. Both methods have given me positive results so far as the occasional presence of microörganisms is concerned, but both are in accord in failing to demonstrate the constant presence of any particular organism. In my culture experiments made in Havana last year the microörganism most frequently encountered was my bacillus *a*, already referred to as found in two out of ten cases in cultures from blood drawn from the heart. Naturally I have given much attention to this bacillus, and it was only after an extended series of comparative experiments that I gave up the hope that it might be concerned in the etiology of the disease under consideration.

These comparative experiments forced me to the conclusion that this is the same bacillus as was found by Emmerich in cholera cadavers at Naples, and that it corresponds with the bacterium coli commune of Escherich.

In my researches by the method of staining thin sections of the tissues hardened in alcohol I have encountered several different microörganisms, but no one of these has been found in a series of cases. One, the bacillus of Lacerda and Babes, I have found only in material brought from Dr. Lacerda's laboratory in Brazil, and in two only out of nine cases represented by material from this source. In one of my Havana cases, in which the material was collected by my friend, Dr. Burgess, in 1887, a long bacillus was found in the kidney, for the most part in the glomeruli. In a case in which I made the autopsy in Havana last spring a micrococcus, grouped in fours, was found in the kidney.

Evidently, if any one of these microörganisms was found in a considerable series of cases the fact would be decidedly significant, and would afford presumptive evidence that the parasitic organism found bore some relation to the morbid process, but even if one and the same microörganism was found in every case the final proof in its etiological import would depend upon its isolation in pure cultures and the production of the characteristic phenomena of the diseases in one of the lower animals, or in the absence of a susceptible animal, in man himself.

The method by cultivation is by far the most reliable for the demonstration of microorganisms which will grow in our culture media, for isolated cocci or bacilli might easily escape observation when present in small numbers, but would serve to start a culture. Thus the bacillus of typhoid fever, which, as stated, is not as a rule found in the blood of the general circulation, and is only found in the spleen in scattered clumps, may be obtained from this organ, in pure cultures, almost without fail, by introducing a small quantity of splenic pulp into a suitable nutritive medium.

Moreover, this method enables us to differentiate microörganisms which look alike, and which by microscopic examination alone it would be impossible to distinguish one from another. This is a fact now well recognized by bacteriologists, but not generally appreciated by microscopists whose researches have been limited to the staining and mounting of sections.

Both methods require skill and practice in the execution, and great caution in drawing conclusions, for there are a thousand traps lying in wait for the explorer in this field of investigation. It is for this reason that pseudo discoveries are so numerous.

Especial care is required in the microscopical examination of stained preparations of yellow-fever tissues. One encounters in the urinary tubules, mingled with the débris of the desquamated epithelium, stained masses of various forms which often closely resemble cocci or bacilli. These I believe to be fragments of nuclear material.

The same material is often massed in the urinary tubules in the form of plugs, which are deeply stained by the aniline dyes.

Again, fragmentation of nuclei of cells still in position may give the impression of a cell containing cocci, and the karyokinetic figures found in the cells, especially in the liver, often resemble bacilli so closely that it is difficult to convince any one not familiar with them that they are not microörganisms.

The "plasma cells" of Ehrlich, also, seem to have as their chief function the role of deluding amateur microscopists into the idea that they have made a discovery. They are often very abundant in the liver and in the kidney of yellow fever cases, and so closely resemble zoogloea masses of micrococci that experienced pathologists have been deceived by them.

In addition to these objects which resemble microörganisms there are dangers from the post-mortem invasion of the tissues when the autopsy has been delayed beyond an hour or two, in the warm climates where yellow fever prevails, or even in the preserving medium or during the process of staining.

My experiments made in 1883 showed that "exposure to 95 per cent. alcohol for 48 hours did not kill the bacteria in broken-down beef tea (old stock)," and pathologists are familiar with the picture presented by the post-mortem invasion of tissues which have been left in alcohol which was not strong enough to preserve them.

Finally, inasmuch as my culture experiments with material collected soon after death from the liver and kidney gave a positive result in a certain proportion of the cases, it is evident that the microörganism most frequently found by this method—my bacillus *a*—should occasionally be encountered in stained preparations.

The possibility remains that by some method of staining not hitherto employed, the specific infectious agent may yet be demonstrated in the tissues; but the fact that my culture experiments with material from the liver and kidney of ten cases failed to demonstrate any such specific microbe is opposed to this view. We may, of course, suppose that the yellow fever germ not only requires special methods yet undiscovered for its demonstration in the tissues, but that it will not grow in the culture media which I have employed in my researches. I would say in reply to this hypothesis that all known pathogenic microörganisms may be demonstrated by the staining methods employed, and that inasmuch as the yellow fever germ appears to find a favorable nidus in filth beds external to the body, I have been inclined to believe that, like the bacillus of typhoid fever and cholera, it is not especially nice as to the character of the medium in which it may develop. However, this may be a mistaken idea, and I propose in my future researches to make use of various culture media not yet employed, and especially to make cultures from the tissues and the excreta in an atmosphere from which oxygen has been excluded; for it may be that like the bacillus of malignant œdema and the bacillus of tetanus the yellow fever microbe is anærobic.

Inasmuch as some of the readers of this report may not have at hand the volume in which the report of my investigations in Brazil and in Mexico is contained, I introduce here a paper read before the College of Physicians of Philadelphia in April, 1888, and published in the "Medical News" of April 28, 1888, in which a summary of the results of these investigations is given:

GENTLEMEN: You are aware that I have been engaged during the past year in an nvestigation of the methods of inoculation practiced in Brazil and in Mexico, by which it is claimed that protection against yellow fever is afforded. The subject is one of such great interest to the medical profession everywhere, and of such importance with reference to the sanitary interests of that portion of the United States subject to invasion by the disease in question, that Congress provided for an investigation of the methods of inoculation practiced in Brazil and in Mexico, in the act

providing for the civil expenses of the Government for the year ending June 30, 1888.

Having been selected by the President to make the investigation referred to, in compliance with my instructions I first proceeded to Brazil for the purpose of investigating the methods of Dr. Domingos Freire, of Rio de Janeiro, and after my return from that country went to Mexico to make a similar research with reference to the value of the method of inoculation practiced by Dr. Carmona y Valle. My detailed report was submitted to the President about a month ago, and by his permission I am now authorized to make public the conclusions reached. As I am about to go to Havana for the purpose of continuing my researches with reference to the etiology and prophylaxis of yellow fever, I am glad to avail myself of the kind invitation of the College of Physicians of Philadelphia, and to present to you, and to the profession generally, the conclusions which I have reached up to the present date.

These conclusions are given as follows in my report, above referred to:

"Facts relating to the endemic and epidemic prevalence of yellow fever, considered in connection with the present state of knowledge concerning the etiology of other infectious diseases, justify the belief that yellow fever is due to a living micro-organism, capable of development, under favorable local and meteorological conditions, external to the human body, and of establishing new centers of infection when transported to distant localities.

"Inasmuch as a single attack of yellow fever, however mild, protects, as a rule, from future attacks, there is reason to hope that similar protection would result if a method could be discovered of inducing a mild attack of the disease by inoculation or otherwise.

"The hypothetical yellow fever germ, multiplying external to the human body in unsanitary places in tropical regions where the disease is endemic, or during the summer months in the area of its occasional epidemic prevalence, establishes infected localities, and susceptible persons contract yellow fever by exposure in these infected areas. We infer, therefore, *a priori*, that the yellow fever germ invades the system by the respiratory tract, by the alimentary canal, or from the general surface of the body, and it should be found in the blood and tissues, or in the alimentary canal, or upon the surface.

"Another possibility presents itself, viz: That the germ multiplying in insanitary localities external to the body produces a volatile poison, which contaminates the air, and that an attack is induced by the toxic effects of this potent chemical poison. The more or less prolonged period of incubation—two to five days—in numerous cases in which the attack has been developed after removal from the infected locality, seems opposed to this latter hypothesis.

"In the light of what is known of the etiology of other infectious diseases, the hypothesis that the germ really finds entrance to the body of the person attacked and multiplies within it, is that which presents itself as most probable, and it hardly seems worth while to consider any other unless this is proved by a complete investigation not to be true. In the latter event we would have to consider the possibility of absorption through the respiratory tract of a volatile toxic agent, or through the skin of a poisonous ptomaine formed upon the surface of the body by a specific micro-organism which does not itself penetrate to the interior.

"Naturally the attention of investigators has first been given to a search for the 'germ' in the blood of those attacked, and in the blood and tissues of the victims of the malady.

"The researches made up to the present time have failed to demonstrate the constant presence of any micro-organism in the blood and tissues of those attacked.

"My own researches, recorded in the foregoing report, show that no such micro-organism as Dr. Domingos Freire, of Brazil, has described in his published works, or as he presented to me as his yellow fever germ at the time of my visit to Brazil, is found, as he asserts, in the blood and tissues of typical cases of yellow fever.

"There is no satisfactory evidence that the method of inoculation practiced by Dr. Domingos Freire has any prophylactic value.

"The claims of Dr. Carmona y Valle, of Mexico, to have discovered the specific cause of yellow fever have likewise no scientific basis, and he has failed to demonstrate the protective value of his proposed method of prophylaxis.

"It is highly important, in the interests of science and of public health, that further investigations be made by more exact methods, which have been perfected since Drs. Freire and Carmona made their researches, and with which they were evidently not familiar."

The failure thus far to find a specific micro-organism in the blood or tissues makes it desirable that a thorough research should be made with reference to the micro-organisms present in the alimentary canal, for it is possible that in yellow fever, as in cholera, the disease is induced by a microörganism which multiplies in this situation. Additional researches are also required before we can say definitely that there is no germ demonstrable in the blood and tissues. Having exhausted our researches by the method of direct examination, and by cultures from blood drawn during life, it is highly desirable that various culture media should be inoculated with material taken, with proper precautions, from the various organs, and at the earliest possible moment after death.*

I can not attempt to give you in detail, at the present time, the evidence upon which the conclusions above stated are based, but it is recorded, in extenso, in my report to the President, and will no doubt be published in due time. I shall, however, take advantage of this occasion to call your attention to some of the principal facts upon which I base the unfavorable opinion expressed with reference to the claims of Dr. Domingos Freire, of Brazil, and of Dr. Carmona y Valle, of Mexico. Both of these gentlemen, as you are aware, have laid claim to priority in the discovery of the specific germ of yellow fever, and both have practiced inoculations with material supposed to contain their "microbe," having in view the production of a mild attack of the disease, and subsequent immunity as a result of this attack.

Dr. Freire has, however, made a greater number of inoculations, and owing to his numerous publications and very positive assertions his claims have received the most attention. My conclusion that these claims are without scientific foundation may seem to you almost incredible, in view of the extended researches which Dr. Freire has made in a locality where yellow fever is endemic, and of the very favorable statistics which he has published in support of the value of his method of inoculation. But I need scarcely remind you that the astonishing development of our knowledge relating to pathogenic microörganisms, which has occurred during the past decade, has been accompanied by numerous announcements of psuedo discoveries in this field of research, and that much confusion has resulted from the premature publication of experimental researches made by enthusiastic investigators not familiar with the exactions of modern science, or with the exact methods by which alone security is offered against such pseudo discoveries.

Dr. Freire insists in all his published works that his cryptococcus xanthogenicus is present in great numbers in the blood and tissues of yellow-fever patients. In his principal work, Doctrine Microbienne de la fièvre jaune, published in 1885, he makes the following statement:

"The microbe xanthogenicus is a cosmopolitan; it does not select its domicile in any organ and has no preference for any organic liquid. We have encountered it with the same characters, the same opulence of proliferation, in the brain, in the muscles, in the liver, in the spleen, in the kidneys, in the lungs, in the blood, in the urine, in the bile, in the vomit, and even in the cephalo-rachidian fluid. However,

* The writer went to Brazil and to Mexico fully prepared to make these experiments, but, unfortunately, was unable to secure any autopsies in either place, and the limit as to time fixed by his orders made it necessary to return to the United States without having made these important researches.

it is necessary to establish a well-drawn distinction as to the blood; the blood of the general circulation shows itself much less charged with the microbes than the blood of the capillaries. Thus, if I could admit any preference on the part of the microbe xanthogenicus, I would say that it pleases itself better in the blood of the capillaries, in the blood which bathes immediately the anatomical elements.

* * * * * * *

"The occasion seems to us a favorable one in order to call attention to some indispensable precautions when the microbes of yellow fever are to be sought in organic solids and liquids. While it is extremely easy to perceive the presence of the microbes of yellow fever in the urine and bile, for example, by placing a drop of these liquids upon a glass slide, covering it with a thin glass cover, and examining it with a power of 450 to 740 or 780 diameters, this proceeding can not be employed when the blood is to be examined. If we proceed in this manner the globules will hide nearly all of the microbes, and the observer will wrongfully conclude that they are very rare in this organic liquid. Not only does the form of the microbe offer a certain resemblance to that of the red corpuscles, but these latter in adhering together envelope the microbian cells, and on the other hand cast upon the cells a jet of light which makes them disappear from the field of the microscope.

"But if we dilute a little drop of blood in a pure solution of sulphate of soda and place it under the objective, the microbes become visible and will appear in considerable quantity.

"It is likewise necessary to make a preparation previously for the examination of the cerebral mass and of the muscles. They should be triturated in a sterilized mortar and mixed afterward with distilled water entirely deprived of organisms, filtered through fine linen which has been passed rapidly through the flame of an alcohol lamp, and afterward a drop of the filtered liquid should be placed upon a glass slide. If we withdraw a little piece of brain or of muscular fiber, even triturated, we will not perceive anything abnormal under the microscope, unless it be the anatomical elements more or less deformed by trituration.

"It is not the same for the liver. It suffices to withdraw a bit of this organ and to crush it between two glass slides. Upon observing it under the microscope we perceive at once a multitude of microbes. This is because in the muscles the microbes are lodged between the fibrillæ and in the substance which surrounds them; and in the brain they are found in the interior of the nerve cells, which must first be destroyed by trituration in order that their parasitic hosts may become visible."

Having made extended researches in Havana in 1879 and during the past year in Brazil and in Mexico, I am able to assert most positively that no such microorganism as Dr. Freire has described is to be found in the blood and tissues of yellow-fever patients. I have examined blood from numerous cases drawn from the finger during life, both in the fresh condition, in preparations stained by various aniline colors, and by culture methods, and I have studied with great care a large number of thin sections of the liver and kidney, stained by the most approved methods, from a considerable number of typical cases of yellow fever, without having encountered the cryptococcus xanthogenicus.

As Dr. Freire asserts that his microbe is especially abundant in the capillary vessels, it should be easily demonstrated in thin sections of the various organs, made *secundem artem*.

As a matter of fact, my researches show that, as a rule, no microörganism demonstrable by known methods of staining is to be found in such sections. But here I must state that in certain cases microörganisms have been found. The one to which the most interest attaches is that described by Babes. This was discovered in 1884, in material sent to Paris from the laboratory of Dr. Lacerda, in Rio de Janeiro. At the time of my visit to Brazil my friend Dr. Aranjo Goes gave me material from nine cases, which had been preserved in Dr. Lacerda's laboratory since the epidemic of 1884, and after my return to Baltimore I examined sections stained by Gram's

method and with Loeffler's alkaline solution of methyline blue from all of these cases. I quote from my report as follows:

"In two of these cases I find in the capillaries of the liver and of the kidneys an organism which has been described by Babes and by Lacerda, who found it in material collected at the same time, and probably from the very same cases as those in which I now find it. This organism is a short bacillus, which occurs in chains, as seen in Fig. 2, Pl. IV.

"In certain places, especially in the kidneys, it is found in the capillaries in great numbers massed together; in other places it is distributed more sparsely, as seen in the figure."

Careful examination of specimens stained with Loeffler's solution shows that the separate elements in these little chains vary considerably as to their length, and that the ends are more deeply stained than the center. This appearance was no doubt observed by Babes, who first described the organism in question, but he has interpreted it differently. He says:

"The filaments appear united and homogeneous with an amplification of 600 diameters, but with a high power (1-12th hom. im., or No. 12 of Verick, which corresponds with the 1-18th of Zeiss) one can assure himself that these filaments are composed of elliptical grains, almost cylindrical, arranged in pairs, forming little groups, in which they are united by an intermediary pale substance. The filaments are thus composed of diplococci, or, if one wishes, of very short rods with terminal spores."

Dr. Lacerda has described the organism referred to as in filaments which branch dichotomously, and believes this branching to be a constant and distinctive characteristic of the parasite which he accepts as the veritable yellow fever microbe. He is without doubt mistaken. The apparent branching of the filaments which he has described and drawn, and which he showed me in some of his preparations at the time of my visit to Rio, is due simply to the accidental juxtaposition of the torula-like chains. He is also mistaken in supposing that this organism is only to be satisfactorily demonstrated by Gram's method of staining. My friend Dr. Goes shared this belief at the time of my visit to Rio, but I demonstrated to him the facility with which the organism may be stained with a solution of methyline blue upon sections which he made for me from material in Dr. Lacerda's laboratory. Since my return to Baltimore I have made numerous sections from the same material (two cases out of the nine), and find no difficulty in staining the organism present in the tissues with methyline blue or with fuchsin. Dr. Goes also supposed that his failure to find this parasite in all of the tissues which he had preserved since the epidemic of 1884 was due to the fact that the tissues had been kept too long. He thought that it was most easily stained in recent tissues, and anticipated that when he had again an opportunity to make autopsies he would encounter this microörganism in the tissues. I shall await with interest his report of his recent researches.

As already stated, I have not been able to find this microbe of Babes in the tissues of six undoubted cases of yellow fever sent to me from Havana, and examined most carefully within six months of the date of the autopsies. Babes himself has renounced the idea that this microörganism bears an etiological relation to the disease under consideration. In the second edition of Les Bactéries he says:

"Since these researches we have had the opportunity to examine several series of sections from yellow fever: First, the liver and kidney of two individuals dead from this malady, collected by Dr. Alvarez, were examined in the Laboratory of Pathological Anatomy of the Faculty of Paris, without any bacteria having been found; second, material from three cases of yellow fever which Koch was kind enough to confide to one of us. In these three last cases, notwithstanding the most scrupulous research and notwithstanding the advice of Koch, it was impossible to find the little chains in the brain, the kidneys, the liver, and the spleen. We must suppose, then, that in yellow fever, as in other infectious maladies, microbes are only found in the parenchymatous organs in certain cases, and not in all. The question whether these

micro-organisms really constitute the cause of the malady, or simply a complication, is, consequently, not resolved." (Les Bactéries, p. 528.)

We would remark that, in view of the negative results attending Babes' more recent researches and our own extended study of the tissues from six typical cases occurring in Havana, there is no good reason for supposing that the above-described microörganism bears an etiological relation to yellow fever. On the contrary, it seems probable that its presence in material from a limited number of cases occurring in Rio is either entirely accidental or is due to a secondary complication, perhaps to some form of septicæmia.

From what has been said and from Dr. Freire's own account of his method of triturating fresh tissues to demonstrate his cryptococcus, the inference seems unavoidable that in his researches made by this method he has mistaken broken-up blood corpuscles and the granular débris of the tissue elements for microörganisms.

In his culture experiments he has, owing to a defective technique, encountered micrococci of various kinds, and one of these, which he brought back with him from Paris in an agar-agar culture, he presented to me as his yellow fever germ.

I brought a pure culture of this micrcroccus with me to Baltimore and have cultivated it during the past winter in various media. It does not correspond in its morphology or mode of development with the *Cryptococcus xanthogenicus*, as described by Dr. Freire in his various published works, and, as stated, is not found in the blood or tissues of yellow-fever patients.

In his principal work, published in 1885, Dr. Freire gives the following account of the morphology of his cryptococcus:

"When we follow with care and attention the march of the development which characterizes the germs which produce yellow fever we acquire the certainty that, commencing to present themselves under the form of little points almost imperceptible, they afterward gradually increase in diameter, until they attain considerable dimensions; so that these little beings, which, at the outset had the aspect of very little grains of sand, not measuring more than 0.001 millimetre to 0.002 millimetre in diameter, arrive, little by little, to such a development that they reach the dimensions of 0.005, 0.007, 0.008 millimetre, and sometimes even more in certain conditions. When they have attained the adult age these cells are broken at divers points, and discharge their contents, composed of spores already formed, mixed with a viscous substance of a yellow color, composed of a pigment and protoplasmic substance, and of the liquids elaborated by the cells."

Even so recently as last year, in an address delivered before the Dosimetric Society of Paris, Dr. Freire repeats this account of the mode of development of his cryptococcus. He says:

"Each adult cell is ruptured in one or several points, and allows the escape of its contents, composed of germs which are to perpetuate the species, and two pigments, one yellow, destined to infiltrate all the tissues, and to produce the icteric color which has given name to the malady; the other, black, insoluble, and destined to be carried along by the circulatory current, producing either capillary obstructions or blood stasis in the parenchyma of the organs."

Now this mode of multiplication is not known among the bacteria, and does not occur in the micrococcus which Dr. Freire placed in my hands as his yellow-fever microbe, which multiplies by binary division and does not differ in its morphology from a variety of microörganisms of the same class, which are extremely common in all parts of the world.

According to Dr. Freire's statement, the agar culture brought by him from Paris came originally from the blood of a patient with yellow fever at the point of death.

Now, as heretofore stated, I have demonstrated by the microscopic examination of numerous specimens that, when proper precautions are taken no microörganisms are found in blood drawn from the finger of yellow fever patients, and that no development occurs in culture media inoculated with such blood. I infer, therefore, that

the micrococci, etc., found by Dr. Freire in his cultures are due to the accidental introduction of microörganisms from without, and especially from the surface of the finger during the collection of the blood. I may remark here that Dr. Freire's cultures in liquid media, which were made before he went to Paris, and which were placed in my hands for examination, were all impure, and contained several different micro-organisms. I suppose that the micrococcus brought back from Paris was isolated while he was there from one of these impure liquid cultures, for I found no evidence that solid culture media had been used in his laboratory in Rio prior to the date of his visit to Paris.

Nor did he use, to any extent at least, the well-known methods of staining bacteria which are recognized by bacteriologists as so essential in the study of these minute micro-organisms. This is shown by the following quotation from his address, delivered in Paris, to which reference has already been made. He says:

"We know that in order to color a microbe it is necessary first to kill it and then to wash the little microscopic cadaver by means of reagents possessing the power of dissolving all matters foreign to its skeleton. At the outset I applied myself to the study of the yellow fever microbe in a fresh state. I fed it with the best food—*les meilleurs engrais*—for the purpose of surprising the different phases of its evolution from its birth to its death.

"Nevertheless, in fault of other accusations, some authors have reproached me with not having colored my microbe. Alas! what a miserable objection. Is it necessary in order to affirm the existence of a microbe which swarms by millions in the urine, in the bile, in the blood, in the tissues, etc., is it necessary to mask them, to disguise them under a costume of carnival, in order to please certain microscopists? M. Pasteur has never colored his microbes, and nevertheless every one admits the existence of the bacillus of charbon, of the corpuscles of pébrine, of the micrococcus of fowl-cholera, etc.

"Do not think, gentlemen, that I fear the application of coloring processes to the search for the microbe of yellow fever. Far from it. In order to show you that the criticism which I have just made is not due to prejudice, I will say to you that such processes have recently been employed upon the yellow-fever microbe with complete success."

A character upon which Dr. Freire insists, even in his address delivered in Paris in April, 1887, is the formation of two kinds of pigment, one yellow and one black. From the first he has affirmed that the black color of the characteristic "black vomit" is not due to the presence of blood changed by the acid secretions of the stomach, as has been generally believed by those physicians who have studied the disease, but that this color is due to a pigment produced by his cryptococcus. Now, I have had the coccus which he gave me as his yellow fever germ under cultivation in various media, during a period of several months, and no black pigment has been produced. On the contrary, the colonies in Esmarch tubes, and stick cultures in gelatine, or in agar, all have a milk-white color. In morphology this micrococcus does not differ from some well-known and widely distributed species. It liquefies gelatine quite slowly, grows readily at a temperature of 18° to 20° C. (64.4° to 68° F.), and, as stated, forms no pigment.

The supposition that the micro-organisms present in Dr. Freire's blood cultures, and in those of various other observers who have discovered yellow fever "germs", came from the surface of the body, and not from the blood, is sustained by recent experimental researches upon the sterilization of the hands, made by Kummell and by Furbringer.

These experiments show that it is not an easy matter to destroy all microörganisms upon the surface of the body by means of a disinfecting solution, and that a simple washing with bichloride solution of one-thousandth does not usually insure sterilization of the hands. Furbringer, after repeated experiments, recommends the following procedure: (1) Remove all visible dirt from the nails. (2) Scrub the hands

with soap and water by means of a brush. (3) Immerse them for one minute in strong alcohol, at least 80 per cent. (4) While still wet immerse them for one minute in a 2 per cent. solution of mercuric chloride.

Less thorough treatment did not in Furbringer's experiments absolutely insure sterilization. In the case of patients in hospital, the difficulty is often increased by the fact that their hands are horny and begrimed with dirt which can only be removed by long scrubbing.

With reference to Dr. Freire's experiments upon animals, I can only say that those performed in my presence failed entirely to demonstrate that the micrococcus brought from Paris had any specific pathogenic power, and that I find no satisfactory evidence in the record of similar experiments contained in his various publications that he has ever transmitted yellow fever to rabbits or to guinea-pigs, as claimed.

I shall quote here a single experiment in which a fatal result occurred, and in which, as usual, Dr. Freire ascribed death to yellow fever resulting from the injection, notwithstanding the fact that the material injected (blood) had been subjected to a boiling temperature for some minutes. My own numerous experiments show that all known micrococci are quickly destroyed by a temperature much below the boiling point of water.*

Dr. Freire's account of this remarkable experiment is as follows:

"We must note that the microbe of yellow fever offers a remarkable resistance to heat. The following experiment furnishes a demonstration of this:

"On the 17th of April, 1883, we subjected to ebullition for several minutes a gramme of blood containing the microbes. We injected it afterward into a guinea pig, which had a temperature before the experiment of 38.5° C. (101.3° F.) in the axillary region. The temperature followed this march the following days:

April 18	39° C. (102.2° F.)
April 19	39°
April 20	39.1°
April 21	39°
April 22	38.7°
April 23	37.4° C. (99.3° F.)

"The animal died during the night of the 23d. Its autopsy showed the characteristic lesions.

"It is necessary to push the temperature beyond 200° C. in order to destroy the toxic energy of the microbe. As we have seen its virulence resists simple ebullition. The microscope has shown that notwithstanding the boiling the micröorganisms retained their ordinary form and continued to execute all of their movements, a proof of their complete vitality." (*Op. cit.*, p. 217.)

I may remark here that the micrococcus presented to me by Dr. Freire as his yellow fever germ is killed by exposure for ten minutes to a temperature of 60° C., and that it has no proper movements.

Dr. Freire's protective inoculations.—Having demonstrated that Dr. Freire's claim to have discovered the specific cause of yellow fever is without scientific foundation, it may be thought that no further demonstration is required in order to show that his preventive inoculations are without value; for these inoculations are said to have been made with cultures containing the attenuated microbe of yellow fever. These inoculations have, however, been made upon so large a scale, and the statistical results, as presented by Dr. Freire, appear so favorable to his method that it becomes necessary to analyze these statistics; and if, as he claims, they establish the fact that the mortality from yellow fever is very much less among those who have been inoculated by him than among non-inoculated persons exposed in the same way, we shall be obliged to concede the value of his method, although the rationale of the

* The Thermal Death Point of Pathogenic Microörganisms, Amer. Journ. Med. Sci., July, 1887, pp. 146–160.

protective influence may not be apparent. In my detailed report I have reviewed at length Dr. Freire's statistics in the light of the facts developed by my personal researches in the city of Rio de Janeiro, where the inoculations were made. I can not attempt to bring the evidence before you at the present time, and I have already stated to you my conclusion with reference to the matter. In support of this conclusion I shall, however, quote a few extracts from my report.

In 1884, Dr. Freire inoculated 418 persons whose names, ages, place of residence, and length of residence in Brazil are given in an appendix to his principal work, published in 1885. In regard to the evidence afforded by these inoculations, I have written as follows:

"We remark, first, that in selecting foreigners and preferably those who had recently arrived in Brazil for his first experimental test as to the efficacy of his protective inoculations, Dr. Freire has given evidence of his confidence in the method of prophylaxis proposed and of an honest desire to demonstrate its value, and nothing could be more fair than his full publication of the names and of the essential facts with reference to these persons.

"I must object, however, to his including in his statistics the names of 37 persons residing in Vassouras, a village which is some 50 miles distant from Rio de Janeiro. Even if these persons had occasion to visit Rio during the epidemic season, as is stated by Dr. Freire, it is probable that they would remain as short a time as possible, and there is no evidence that they were fairly exposed to the epidemic influence. Moreover, if any of these persons had contracted yellow fever as a result of a visit to Rio their names would not appear in the mortality list of this city, but in those of Vassouras, which are not given. The latter objection applies also to 14 persons among the vaccinated whose place of residence is Nictheroy, a town upon the bay of Rio de Janeiro, which is the capital of the province of the same name; two persons vaccinated at Tijuca, and one on board the bark *Flive*, two at Pavina, and three at Serraria must also be excluded. This reduces the number of vaccinated persons within the city limits to 355, and of this number a certain proportion no doubt left the city soon after being vaccinated, and before any exposure worthy of consideration in a test of this kind had occurred."

Dr. Freire admits that "during the epidemic season a great number of the vaccinated were attacked by the malady," but claims that these attacks were of a mild character, yet he gives us the names of seven vaccinated persons who died from the disease. This list has been added to by some of Dr. Freire's confrères, as will be seen by the following translation of a letter published in one of the newspapers of Rio, and bearing date May 5, 1887. This letter is signed by Dr. Araujo Goes, at present a member of the Central Board of Health, and a gentleman whose statements are worthy of the fullest confidence:

"My letter to the Imperial Academy of Medicine having been published, it now behooves me to publish the statistics relating to the vaccinations on Morro da Viuva.

"One fact seems to me to be definitely demonstrated, that is the *worthlessness of Dr. Freire's vaccination*, as is well known to the medical profession of this city.

"A year ago I wrote the following:

"The want of skill which he displayed in his first experiments, the false conclusions which he has drawn therefrom, and the thoughtless precipitation with which he has hastened to make known incomplete results without accompanying them with a single qualifying remark vitiate all the methods to which he may hereafter resort to corroborate his statements. (Journal do Commercio, April 20, 1883.)

"The mortality among the persons vaccinated on Morro da Viuva furnishes one more proof that I was right in saying this, as I now proceed to demonstrate.

"There were vaccinated in this district 60 persons.

"Sixteen removed shortly after the commencement of the epidemic, and 44 remained exposed to its influence. Of these 22 had yellow fever, 9 of whom died.

"The following is a list of the vaccinated persons who had the fever, the names of those who died being marked with an asterisk:

Antonio de Oliveira.*
Antonio Bento da Silva.
Albino Francisco Maia.
Jose da Silva.
Joaquin Pereira da Souza.*
Joaquin Gomes de Azevedo.
Joaquin Ferreira Tolho.
Jose Scabra dos Santos.
Jose de Souza Ferreira.
Jose Gomes de Azevedo Junior.
Jose Farinha.
Manoel Joaquin Pereira Lopes.
Manoel Gomes de Azevedo.
Manoel Antonio.*
Seraphim Goncalves Raymundo.
Manoel Simoes.*
Thome Simoes.
Manoel da Silva Alves.*
Antonio Pereira Neves.*
Joaquim Martins Pinheiro.*
Joaquim Antonio dos Santos Cardoso.*

"Consequently, of the 44 vaccinated persons who remained in the locality, 22, that is 50 per cent., had the yellow fever.

"Of the 22 patients, 9 died, that is 40.9 per cent.

"In the Jurujuba Hospital, which receives scores of patients already dying or in the third stage of the disease, the mortality is only 21 per cent."

After reviewing all of the evidence obtainable relating to the inoculations practiced in 1883 and 1884, I say:

"The evidence above recorded seems to the writer to be convincing as to the complete failure of Dr. Freire's proposed method of prophylaxis as practiced in 1883 and in 1884. We can not, however, leave the question here, inasmuch as a modification of the method was adopted in 1884, and a large number of persons have been since inoculated by this modified method. Dr. Freire says in his report under consideration: 'I have employed in nearly all of the vaccinations the endermic method, and it is only recently that I have injected into twenty persons the same cultures by the hypodermatic method. * * * I shall hereafter give the preference to the last mentioned method, because one is more sure that the liquid employed goes to exercise its preservative influence.'"

In 1885 Dr. Freire resumed his inoculations on a larger scale, but instead of selecting unacclimated strangers, those inoculated were for the most part natives of Brazil, or Portuguese who had lived for a number of years in Rio and who had passed through one or more epidemics. A considerable number of negroes were also inoculated and included in the statistical tables. With reference to Dr. Freire's statistics for the year 1885 I quote from my report as follows:

"Dr. Freire has omitted to state one very important fact with reference to the vaccinations practiced during the period included in this tabular statement. The date of the vaccinations is not given. Fortunately I am able to supply this omission from his journal containing the names of the vaccinated, which he kindly placed in my hands during my stay in Rio. I find from this record that the inoculations were practiced as follows:

January	392
February	342
March	611
April	139
May	273
June	813
July	481

"Now it is well known that June and July are months during which yellow fever does not prevail in Rio, and that in fact the month of May furnishes, as a rule, but few cases.

"The exposure even in an epidemic year amounts to very little during the months of May, June, and July, and may be considered practically nil in a year like 1885,

when the whole mortality was only 278 in a city of 400,000 inhabitants. But Dr. Freire has included in his list 1,294 persons who were vaccinated during the healthy winter months of June and July, and who presumably had been exposed during the preceding comparatively unhealthy months of January, February, March, and April. If these 1,291 individuals were protected from an attack of yellow fever by the inoculation practiced in June or July, what protected them from being attacked during the preceding months when yellow fever was prevailing to some extent?

"We remarked with reference to those persons selected for vaccination in 1883 and 1884 that 'in selecting foreigners and preferably those who had recently arrived in Brazil for his first experimental test of the efficacy of his protective inoculations Dr. Freire has given evidence of his confidence in the method of prophylaxis proposed and of an honest desire to demonstrate its value.'

"In his inoculations practiced in 1885 we no longer find any evidence of such selection, and so far as we can judge the vaccinated persons simply represent the average population of the city of Rio. It is well known that this population includes a large number of persons of foreign birth and especially of Portuguese. The whole foreign-born population probably does not fall below 100,000 persons, but I have not been able to obtain any exact statistics with reference to this point. Dr. Freire vaccinated 2,186 natives and 865 foreigners. Let us assume for the present that the 1,760 persons vaccinated by him during the months of January, February, March, April, and May were comparable, so far as the susceptibility to yellow fever is concerned, with 300,000 of the population of Rio. In this estimate we exclude 100,000 of the population on the supposition that this number may have enjoyed immunity as a result of having suffered an attack of the disease, 278 deaths in a population of 300,000 gives less than 1 death per 1,000, and there should not have been over 2 deaths among the 1,780 persons inoculated by Dr. Freire during the months of yellow fever prevalence.

"Let us look at the matter in another light. Dr. Freire gives the following table, showing the length of residence in Brazil of the foreigners inoculated in 1885:

For a few days	26
For a few months	71
From 1 year to 1 year and a half	69
Two years	107
Three years	98
Four years	126
Five years	103
On board ship (de passage)	9
More than 5 years	256
Total	865

"These figures also include the foreigners vaccinated in the healthy months of June and July and those temporarily in the city (a few days, 26, on board ship, 9), but they will serve our present purpose, which is to call attention to the fact that 759 of the total number given had been in Brazil (and presumably in Rio) more than a year and a half and had consequently passed through the preceding epidemic year (1884) without contracting yellow fever. If these persons resisted yellow fever during an epidemic, in which the number of deaths amounted to 1,597, how can it be claimed that they are protected by a vaccination made in 1885, when only 237 deaths occurred, scattered about (sporadic) in a city of 400,000 inhabitants? The same argument applies with greater force to the 359 foreigners inoculated, who had resided in Brazil for 5 years and above (5 years, 103, more than 5 years, 256). Unless the list includes persons who had already suffered an attack of yellow fever, these individuals had passed through the epidemic of 1880 (1,623 deaths), as well as that of 1883 and 1884, without contracting the disease, and we can hardly ascribe their immunity in the comparatively healthy year, 1885, to Dr. Freire's inoculation."

I pass now to the year 1886, during which Dr. Freire inoculated 2,763 Brazilians

and 710 foreigners, again including in his statistical tables those vaccinated after the epidemic season had passed. In reviewing these statistics I remark as follows:

"We have quoted this last report of Dr. Freire in extenso in order to do him full jutice by allowing him to state his own case. We shall now proceed to show that his statistics are fallacious, and that the percentage of mortality among the vaccinated, which he finds to be ten times less than among the non-vaccinated, results from a misuse of the statistical method and from a number of factors, which are favorable to Dr. Freire's statistics as he has stated them, but not to a fair test of his method of prophylaxis.

"In the first place we would call attention to the fact that while during the comparatively healthy year, 1885, the immunity among the vaccinated of that year is said to be complete (see report of 1885), the number of deaths during the epidemic year which followed is stated by Dr. Freire himself to have been 8. Taking all of the vaccinated of the two years, and without making any allowance for the considerable number of persons vaccinated who had, no doubt, left the city before the epidemic of 1886 occurred, Dr. Freire, with a total of 6,524 vaccinated, and a total of 8 deaths, makes the proportion one per thousand. This is equivalent at the outset to an addition of 1,476 person to the number vaccinated, who being imaginary persons and not having been exposed to the epidemic influence simply aid in rounding up the general percentage of mortality in Dr. Freire's favor to the even figure of one per thousand. This is but one of many factors which go to make this favorable showing. Reference to Dr. Freire's MS. journals, which he kindly placed in my hands, shows that of the total number vaccinated during the two years, 4,465 were vaccinated prior to the epidemic of 1866; that is to say, before the 1st of January, 1886. How many of these left the city before the ontbreak of the epidemic, how many were only temporarily in the city when vaccinated, how many died from other diseases I can not say; but it is a significant fact that of the 3,051 vaccinated prior to August, 1885, Dr. Freire has only one fatal case to report, while out of 460 persons vaccinated in January and February, 1886, he reports 5 deaths, a mortality of more than 1 per cent., which he gives as the general mortality among the non-vaccinated. This is not apparent from his own statement of the case, but is nevertheless true, as I shall proceed to show. In his report, which we have just given in full, he does not give the date of the vaccination of these individuals, but upon referring to his MS. journals for 1886 I find that No. 3 of his list, José, son of José da Costa Vieira, was vaccinated February 12, 1886; No. 4, Paschoal Ruffino, on the 6th of February, 1886; No. 5, Henri Constance, on the 1st of January, 1886; No. 6, Fernando Argenteiro, on the 20th of February, 1886, and No. 7, Antonio Saraiva, on the 12th of February, 1886. The same MS. record for 1886 shows that during these two months—January and February, 1886—the total number vaccinated by Dr. Freire was 460. That is to say the mortality among those vaccinated during these two months was more than 1 per cent. On referring to the mortality list of the city for the same two months I find the total number of deaths to have been 369, which in a total susceptible population of 160,000 (Dr. Freire's estimate) would give a mortality of 1 in 436."

Time will not permit me to extend any further this analysis of Dr. Freire's statistics, and I must refer you to my complete report for additional details, and for an account of my personal investigations in Rio de Janeiro. Nor can I occupy any further time in an account of the inoculations practiced by Dr. Carmona y Valle, of Mexico, and of his alleged discovery of the specific cause of the disease under consideration. A simple perusal of Dr. Carmona's published work is sufficient to convince any competent bacteriologist that owing to a defective technique and inexperience in bacteriological researches, he has fallen into serious errors of observation and of inference, and that his supposed discovery has no scientific basis.

From what has been said, it will be seen that the question of yellow fever etiology is still unsettled, and that it remains, in fact, just where it was left by the commis-

sion sent to Havana in 1879 by the National Board of Health. The researches made by myself as a member of that commission showed that in yellow fever there is no microörganism present in the blood of the sick demonstrable by the highest powers of the microscope. This conclusion is supported by my more recent researches by the method of cultivation. Before, however, abandoning all hope of finding a specific "microbe" in the tissues, I desire to make cultures from the various organs, obtained at the earliest possible moment after death. Unfortunately, the opportunity did not offer itself for this important experiment during my visit to Brazil and to Mexico last year; but I hope to have the desired opportunity in Havana during the present summer, and shall also give special attention to a search for the yellow-fever germ in the alimentary canal, where it may possibly be located, as is the case in cholera. It is true that the clinical history of the disease does not especially point to this location; but in a research of this nature it will not answer to reject any possible hypothesis because of preconceived opinions, and inasmuch as the present state of science justifies a belief in a specific microörganism as the essential agent in the etiology of this disease, it is imperative that the investigation be continued until success crowns our efforts.

In a report submitted to the President in March, 1888, and published in the annual volume of the Marine Hospital Service for 1889, full details are given of my investigations made in the City of Rio de Janeiro, and those who desire fuller information with reference to Dr. Freire's "protective inoculations" are referred to this report.

The summary statement above quoted, which was published in the Medical News of Philadelphia, gave great offense to Dr. Freire and led to the publication of a pamphlet entitled "La Mission de Dr. Sternberg au Brésil," in which I am accused of incompetence, myopia, and improper conduct in the prosecution of the mission with which I was charged by the President of the United States.

With reference to my eye-sight I may say that I have never been myopic, but that on the contrary I wear glasses to correct the presbyopia common to persons of my age.

Whether Dr. Freire's other charges have any better foundation I must leave to the judgment of those who may think them worthy of attention.

To answer the violent attack which he has made upon me in detail would be a waste of time, but I take the liberty of introducing here the postscriptum to my published report above referred to, in which I have shown how little foundation there is for his claim that his alleged discovery has been confirmed by other observers.

POSTSCRIPTUM.

BALTIMORE, *September* 23, 1889.

In his attempt to neutralize the force of my evidence and to establish his claim to have discovered the specific microbe of yellow fever, Dr. Domingos Freire has referred to the observations of Babes, of Finlay, and Delgado, of Gererd and of Rangé, as confirming his own. As a matter of fact, the observations of the gentlemen referred to give no support whatever to this claim, inasmuch as none of them have described anything corresponding with the *Cryptococcus xanthogenicus*, or even with the micrococcus which he presented to me as his yellow fever germ.

Thus the microörganism found by Babes in material sent to him from Dr. Lacerda's labaratory in Rio de Janeiro is a short bacillus, arranged in chains, and not a micrococcus. Babes himself has reported his failure to find this bacillus in material from other sources, and his researches show the absence of Freire's micrococcus in the material examined by him, as this is easily stained by the aniline colors, and if present could not have escaped the observation of so accomplished a microscopist and bacteriologist. In the second edition of "Les Bactéries," Babes says:

"Since these researches we have had the opportunity to examine several series of sections from yellow fever: First, the liver and kidney of two individuals dead from this malady, collected by Dr. Alvarez, were examined in the Laboratory of Pathological Anatomy of the Faculty of Paris, without any bacteria having been found; second, material from three cases of yellow fever which Koch was kind enough to confide to one of us. In these last three cases, notwithstanding the most scrupulous research and notwithstanding the advice of Koch, it was impossible to find the little chains in the brain, the kidneys, the liver, and the spleen. We must suppose, then, that in yellow fever, as in other infectious maladies, microbes are only found in the parenchymatous organs in certain cases, and not in all. The question whether these micro-organisms really constitute the cause of the malady, or simply a complication, is, consequently, not resolved."

The extended researches of my friend, Dr. Carlos Finlay, of Havana, also give no support to Freire's claims inasmuch as the micrococcus in tetrads, which has especially engaged his attention and which for a time he believed to be the specific etiological agent in the disease under consideration, is entirely distinct from the micrococcus of Freire. Finlay's *Micrococcus tetragenus febris flavæ*, which I have called *Micrococcus tetragenus versatilis*, a name which he accepts, is a large coccus in tetrads, which differs essentially, both in its morphology and in its growth in culture media, from the micrococcus of Freire. This I can assert most positively, as I have had authentic cultures of each, given me by the gentlemen themselves, under continuous observation for nearly two years.

Moreover, I have made extended culture experiments in Havana during the past two years, which show conclusively that neither of these micrococci is present in the blood of yellow-fever cadavers, withdrawn from the heart or liver shortly after death. In one case only out of thirty-five autopsies in which I have made cultures from the liver, I have obtained the "tetragenus" of Finlay, and I have not encountered the micrococcus of Freire in a single instance. On the other hand, I have obtained both of these cocci in cultures from the surface of the body of patients in the hospitals of Havana, and the "tetragenus" is one of the most common microörganisms encountered in such cultures, whether made from the surface of yellow-fever patients or those suffering from other diseases.

With reference to the observations of Gererd it is evident from his own account that if he encountered micrococci they were associated with spore-forming filamentous bacilli, and that he was entirely unfamiliar with this class of microörganisms. As he is not known as a bacteriologist and has not given a detailed account of his methods of research, no scientific value can be attached to his observations. In a translation of his report made by Dr. Wolfred Nelson and published in the Canada Medical Record of July, 1886, I find the following account of the morphology of the micro-organisms encountered by him:

"In the month of June, 1882, in a report to the superior agent of the Interoceanic Canal Company, resident in the city of Panama, South America, I had the honor to inform him that I had found in the blood of yellow fever patients some microscopic organisms, some filiform, others resembling a string of beads (chaplets), and lastly brilliant little bodies; that the organisms were constant in appearance and could thus serve as elements for diagnosis.

"After some trials and a great many failures, I succeeded in isolating the microbes, and obtained them in great quantity without the human body by artificial cultivation, in liquids suitable for their nutrition and reproduction.

"I was then enabled to study the mode of existence of the microbes. If one observes the filiform bodies attentively for a given time he perceives in their transparent and homogeneous substance a series of small corpuscles that reflect light more than the other parts of the microbe. Little by little these corpuscles arrange themselves around a central axis or core, giving the organism the appearance of a string of beads, chaplet. (This French word signifies the string of beads 'told' by devout Catholics while praying.) Soon other changes follow, the string-like formation separates, and in place thereof nothing remains but a mass of brilliant little points. The size of the little points is about the thousandth of a millimetre. These corpuscle germs have great resistance. *They do not perish by drying, and can after many years serve to propagate the disease by regenerating the filiform bodies when placed under favorable conditions.*"

Compare this with Dr. Freire's account of the morphology and mode of development of his *Cryptococcus xantrogenicus*. In his principal work, published in 1885, he says:

"When we follow with care and attention the march of the development which characterizes the germs which produce yellow fever, we acquire a certainty that, commencing to present themselves under the form of little points almost imperceptible, they afterward gradually increase in diameter until they attain considerable dimensions; so that the little beings, which at the outset had the appearance of little grains of sand, not measuring more than 0.001^{mm} to 0.002^{mm} in diameter, arrive little by little to such a development that they reach the dimensions of 0.005, 0.007, 0.008^{mm} and some times even more in certain conditions. When they have attained the adult age these cells are broken at divers points and discharge their contents, composed of spores already formed, mixed with a viscous substance of a yellow color, composed of a pigment and protoplasmic substance, and of the liquids elaborated by the cells."

In an address delivered in Paris in 1887, Dr. Freire repeats this account of the mode of development of his cryptococcus. He says:

"Each adult cell is ruptured at one or several points and allows its contents to escape, composed of germs which are to perpetuate the species, and two pigments—one yellow, destined to infiltrate the tissues and to produce the icteric color which has given name to the malady; the other black, insoluble, etc."

Dr. Rangé, a medical officer of the French navy, whose researches have been repeatedly referred to by Dr. Freire as confirming his own, says in his report:

"Unfortunately for our researches we did not possess high powers. I could not exceed 540 diameters, and I had no coloring matters for isolating the microbe in the blood according to the method of Ehrlich; therefore I only give these details with reserve. The figured elements which we have drawn were met with in the black vomit of man, the contents of the stomach of guinea pigs, in the cultures of blood and in condensed watery vapor, but in less number. They are agglomerations of cells, some round with a central nucleus; beside these, and with a more considerable development, we met with elliptical cells having the dimensions of a blood globule, and with a nucleus near one of the extremities of the greater diameter. These cells were found in groups of two or three, joined by the extremity containing the nucleus. This approached the periphery little by little; at this moment one observed a slight swelling, a sort of bud, which separated from the cell which had given it birth. Beside these elements one finds others in the form of rods, large and short, not branched. These bacilli sometimes contain granules. We believe that they come from the elliptical cells, for we have followed under the microscope the phases of their transformation. But the absence of a didactic treatise, the absolute absence of bibliographic resources, prevents us from making any positive affirmation with reference to these micro-organisms."

Notwithstanding the very just conclusion above reached, Dr. Rangé, at the end of his memoir, says:

"In uniting these various results, shall we conclude that there is a bacillus of yel-

low fever, bacillus icteroid, and that it is possible to find a vaccine? We believe it without affirming it."

The above quotations will suffice to show any well-informed bacteriologist that the claim of Dr. Freire does not receive any support from the observations of the gentlemen mentioned, whereas several competent bacteriologists have reported their failure to find his "cryptococcus" or any other micro-organism in the blood.

Dr. Paul Gibier, who went to Havana in the expectation of finding what Freire had described, made researches by approved bacteriological methods and reports an entirely negative result. In a communication to the French Academy of Sciences he says:

"HAVANA, *January* 22, 1888.

"At the commencement of the year 1887 Dr. Domingos Freire, professor in the faculty of medicine of Rio de Janeiro, came to Paris in order to present to the scientific public his studies upon yellow fever. M. Fréire was presented to me by Dr. Rebourgeon, who had studied this malady with him in Brazil. The laboratory of comparative pathology of the museum was open to these savants, who resumed the experiments the results of which had previously been published by M. Freire. I was requested by M. Freire to examine the cultures which he had brought with him and to treat them by the new bacteriological methods, which had not yet been applied in his researches. After these investigations, made in common, M. Freire had the kindness to associate me in a communication which he made in his own name and that of Rebourgeon to the Academy of Sciences during the month of March, 1887.

"Since, and as a result of this communication, I received from the minister of public instruction the mission to go and 'study yellow fever in the countries where it prevails habitually, and the prophylactic measures which may be opposed to this malady.' In the early part of November, 1887, I disembarked at Havana, where the yellow fever still shows itself at this epoch in the so-called sporadic form.

"I give as succinctly as possible the results of my first investigations, which were made in the hospitals of Havana.

"*November* 16.—Among several cases of yellow fever I chose that one which appeared to me to be the most grave in order to collect blood and urine. Fifth day of sickness: Fever, albuminuria, black vomit, etc. Fatal case.

"*November* 17.—In an autopsy practiced about 8 hours after death, I collected blood from the left ventricle and from the right auricle. * * *

"*November* 27.—Among several cases examined I collected, from the most severe, blood and black vomit. Fatal case.

"*December* 14.—Case in fourth day of the disease: Albuminuria; collected blood, urine, and black vomit. Recovered.

"*December* 22.—Clinical examination of a severe case: Abundant black vomit, buccal hemorrhage, etc.; 23d, autopsy of this case made 2 hours after death; collected blood from the heart. * * *

"In order to avoid useless repetition I will detail in a general way the methods pursued in the examination of the liquids collected, with the precautions usual in bacteriological researches.

"In each case several preparations of blood were examined in a fresh condition, then dried and stained; the same method was pursued with the urine and the black vomit.

"Inoculations, by numerous punctures, were made in agar-agar jelly with blood, urine, bile, and serum from the pericardium. * * * Numerous thin sections of the various organs were also made; these were stained with a view to demonstrating the presence of microbes.

"*Results obtained.*—I am obliged to confess here, however much it may cost me, that my results contradict in an absolute manner the facts advanced by M. Domingos Freire, from whom I have the regret, as well as the duty, to separate myself.

"*The blood.*—In a great number of preparations, fresh or colored, it has been impossible for me to verify the presence of micro-organisms. The cultures, repeated a great number of times, remained sterile.

"*The urine*, treated in the same manner as the blood, has constantly given a negative result.

"The pericardial liquid and the bile, like the blood and urine, did not contain micro-organisms. I have found that even in the gravest cases seen by me the blood examined by the microscope did not present any appreciable trace of alteration of its elements.

"The numerous sections which I have made of the different viscera also have failed to show me the presence of microbes."

Dr. D. Tomayo, of the bacteriological laboratory of the "Crónica Médico-Quirurgica," of Havana, has also reported a negative result in his repeated examinations of blood drawn from the finger during life. His evidence is valuable both because he is a competent and conservative bacteriologist, having been instructed in the methods of research in Pasteur's laboratory in Paris, and also because he gives a detailed account of his method of collecting blood, which shows that he took extraordinary precautions to prevent accidental contamination of his cultures.

We quote from his paper published in 1888 as follows:

"*Analysis of the blood in yellow fever.*—In collecting blood we have pursued the following technique: We have carefully washed the finger with soap and water; after that we have passed it through a hole made in a piece of impermeable linen a foot square; we have then washed the finger with ordinary alcohol, and afterwards with a solution of bichloride of mercury, and finally with a mixture of ether and absolute alcohol. We have also washed the isolating linen with a solution of bichloride and have covered it with a layer of glycerine. This done we isolated the finger in a little glass tube (cloche de cristal), which had been washed with the sublimate solution and well heated. By this complicated technique we have endeavored to thoroughly cleanse the skin, to remove all grease and every microbe which might be in its folds and furrows, and thus to avoid infection by atmospheric germs. Then we sterilized a lancet in the flame of an alcohol lamp, punctured the skin, and allowed the first drop of blood which presented itself to escape, using only those drops which came later, and that at the moment of their appearance. Following this proceeding we have made *ensemencements*, either with the platinum needle or with sterilized pipettes, in agar-agar jelly, in peptonized gelatine, and in bouillon. We have also examined the blood collected in this way in the artificial serum of Malassey, filtered and sterilized, and lastly dried by the method of Koch. The patients from whom we obtained blood were in the third and the sixth day of the disease; in another case the blood was collected at the moment of death. Up to the present time the cultures in agar-agar, in gelatine, and in bouillon contained in Pasteur flasks have remained sterile."

THE MICROCOCCUS TETRAGENUS FEBRIS FLAVÆ OF DRS. FINLAY AND DELGADO.

My friend, Dr. Carlos Finlay, of Havana, is a most enthusiastic and industrious investigator, but like many other pioneers in bacteriological research at a distance from the centers where the modern exact methods had their origin, at the time of making his first publications he was not familiar with the methods of isolating and differentiating microörganisms, and fell into the usual and almost inevitable errors of inference as to the source and genetic relations of the various microörganisms encountered by him in his earlier researches. He has since made himself familiar with the methods referred to, and no longer insists upon the etiological relation of this micrococcus to the disease under consideration. I give below a letter received from him shortly before my departure from Havana:

"HAVANA, *August* 29, 1889.

"MY DEAR DOCTOR: I send you a copy of the résumé of our investigations during the year, May, 1888-'89, which Dr. Delgado and myself presented at the beginning of the year. You will see that we did not claim to have demonstrated that our tetragenus was the actual germ of yellow fever, but merely that in our recent investi-

gations carried out with methods which we deemed to be reliable, we had again found the same microörganism in yellow fever finger blood and in blister serum, and also in cadaveric products of two yellow fever autopsies. We likewise expressed the hope that you would undertake comparative experiments in order to determine, first, whether it was a fact that by the culture methods which we had described our tetragenus could be demonstrated in most of the products collected during life from yellow fever patients; and, second, whether that microörganism is exclusively found in such patients.

"I am aware that the results of three samples of yellow fever blister serum and seven samples of blister serum from acclimated subjects have given a negative answer on the second point. Yet I can not wholly divest myself of the suspicion that the greater frequency with which we have found the tetragenus in our yellow fever cultures (from material collected during life) may have some significance, even admitting, as I do, that before any etiological importance could be claimed for it, quite a number of serious objections would have to be encountered, besides showing that it is not to be found in localities where yellow fever is unknown.

"I remain, my dear doctor, yours very faithfully,

"CARLOS FINLAY.

"Dr. G. M. STERNBERG, *U. S. Army, Havana.*"

As already stated, I have found this "tetragenus" of Drs. Finlay and Delgado to be one of the most common microörganisms upon the surface of the body of patients in hospital with various diseases, in Vera Cruz and in Havana. I also obtained it in specimens of blister serum collected by Drs. Finlay and Delgado from a case of brain disease, and from a case of skin disease, both of which cases were isolated from any association with yellow fever patients. The blister serum was collected from these cases and brought to my laboratory for the purpose of making a comparative research. I also frequently encountered colonies of the tetragenus in my laboratory in Esmarch tubes which had been inoculated with pure cultures of other microörganisms, showing that it is quite a common atmospheric "germ" in the City of Havana.

In a recent report made by Assistant Surgeon J. J. Kinyoun to the Supervising Surgeon-General of the Marine Hospital Service is stated:

"The microörganism described by Dr. Carlos Finlay (vide London Lancet, September 1, 1887) has been under observation during the past year.

"Experiments made upon various animals gave no results. Later, while the observations on malarial fever were under way, this organism was discovered upon the skin of a majority of the patients suffering from malarial fevers, the patients hailing from Portland, Me., to Vera Cruz, Mex."

During the past year Dr. Freire has again resumed his "vaccinations" on a large scale, and he has recently published a *brochure* in which, as heretofore, he claims wonderful success for his method.

His pamphlet is entitled "*Statistique des vaccinations au moyen des cultures du microbe attenué de la fièvre jaune*" (Rio Janerio, 1890).

No doubt some of those into whose hands this pamphlet falls will be convinced by the array of figures presented, that a wonderful discovery has been made and that Dr. Freire is, indeed, as his friends have claimed, the Pasteur of Brazil. But when his statistics are regarded in the light of the facts developed during my visit to Brazil, and recorded in my published report, it will be seen that they have no scientific value.

In the first place there has been no veritable discovery of the specific

germ of yellow fever and consequently there is no "attenuated virus" with which to vaccinate. So long as Dr. Freire's vaccinations were made with impure cultures it was possible that by accident the veritable yellow fever germ was present. But it is certain that the micrococcus which he presented to me as his yellow fever microbe, his *Cryptococcus xanthogenicus*, has nothing to do with the etiology of this disease. A careful bacteriological study of forty cases, made in Havana since my return from Brazil, enables me to affirm this in the most positive manner. There is then no scientific basis for his wholesale inoculations. And, when his statistics are considered in the light of the facts heretofore referred to, they give no substantial support to his claims.

I shall consider here only that portion of Dr. Freire's latest publication which relates to vaccinations made in the city of Rio de Janeiro. In this city the deaths from yellow fever are recorded and published by the health authorities, and we may accept the figures given by Dr. Freire as corresponding with the official report: He says:

> Between the 1st of March and the 30th of June, 1889, 2,407 persons died of yellow fever (including the deaths at the Jurajuba Hospital), 21 of whom had been vaccinated; that is to say that 2,386 non-vaccinated persons succumbed to the disease (1,606 in the city, 800 at Jurajuba, in all).

Now the total population of Rio is estimated at 400,000. Let us suppose that 100,000 of this population enjoys protection from having suffered an attack of the disease; we have left 300,000 persons, who may fairly be compared with those vaccinated by Freire, who were exposed during the epidemic, and among whom the mortality was 1 in 125 and a fraction ($\frac{300000}{2386} = 125.7$).

Among the 2,087 "vaccinated" there were, according to Dr. Freire, 21 deaths, that is, 1 in 99 and a fraction ($\frac{2087}{21} = 99.38$). It will be seen that this comparison is not at all favorable to Dr. Freire's method. But no doubt he will claim that the comparison is unfair, and that the 2,087 vaccinated by him represent a greater proportion of susceptible persons than the 300,000 of the population with whom we have compared them. Let us then deduct another 100,000 of the population, considering one-half as protected by a previous attack or long residence in the city. The remaining moiety includes all the foreigners residing in the capital city, all Brazilians from other parts of the Republic, all the children below 3 years of age, who, according to Freire, are to be classed with strangers as to susceptibility.

The ratio of mortality is now but little above that among the vaccinated, viz, 1 in 83 and a fraction ($\frac{200000}{2386} = 83.78$).

But in this comparison we have ignored some very important factors which are in favor of Dr. Freire's statistics. A large number of the deaths no doubt occurred among strangers who did not belong to the population of the city, and especially among the sailors on foreign vessels arriving during the epidemic, who are commonly sent to the Jurajuba Hospital when taken sick. On the other hand, we have no definite

information as to the date when the vaccinations were practiced, or the exposure before and after vaccination. In the statistics of previous years a very considerable number of persons were vaccinated after the epidemic had terminated. That is, persons who had passed through the epidemic season without contracting the disease were vaccinated and counted among those supposed to be protected from an attack by this procedure. Evidently the later in the epidemic the vaccinations were practiced, the less value can be accorded to the subsequent exposure as a test of protection. Previous exposure without being taken sick is, on the contrary, evidence of comparative insusceptibility.

To put those vaccinated on the same footing with the 200,000 of the population of Rio with whom we have compared them, they should have been vaccinated at the outset of the epidemic, and exposed in the infected city throughout the epidemic season. How many were vaccinated when the epidemic had commenced to decline, or after it had practically terminated? How many left the city soon after being vaccinated?

These are questions we can not answer. But what has already been said will suffice to show that the results obtained during the recent epidemic in the city of Rio do not give any substantial support to Dr. Freire's claims.

To give completeness to this report, as a convenient book of reference, I introduce here a systematic account of the disease to which it relates, written in 1888 for Wood's "Reference Handbook of the Medical Sciences" (William Wood & Co., publishers, 56 and 58 Lafayette Place, New York). This article is reproduced here by permission of the publishers.

YELLOW FEVER.

DEFINITION.

A specific infectious disease, contracted by exposure in infected localities; characterized by a single febrile paroxysm of short duration (2 to 5 days), by the presence of albumen in the urine, an icteric color of the skin, and a tendency to passive hemorrhages from mucous surfaces—especially from the stomach—producing "black vomit."

HISTORY AND GEOGRAPHICAL DISTRIBUTION.

The geographical range of yellow fever is more restricted than that of any other acute infectious disease, and within the area of its prevalence it is essentially a disease of the littoral, and especially of seaport cities. While occasional epidemics have occurred upon the southwest coast of the Iberian peninsula, the disease, as an epidemic, is unknown elsewhere in Europe, and there is no evidence that it has ever invaded the great and populous continent of Asia. In Africa it is limited to the west coast. In North America, although it has occasionally prevailed as an epidemic in every one of our seaport cities as far north as Boston, and in the Mississippi Valley as far north as St. Louis, it has never established itself as an epidemic disease within the limits of the United States. Vera Cruz, and probably other points on the Gulf coast of Mexico, are however at the present time, endemic foci of the disease. In South America it has prevailed as an epidemic at all of the seaports on the Gulf and Atlantic coasts, as far south as Montevideo and Buenos Ayres, and on the Pacific along the coast of Peru.

The region in which the disease has had the greatest and most frequent prevalence is bounded by the shores of the Gulf of Mexico, and includes the West India islands. Within the past few years yellow fever has been carried to the west coast of North America, and has prevailed as an epidemic as far north as the Mexican port of Guaymas, on the Gulf of California.

The idea that yellow fever may originate *de novo* within the area of its occasional prevalence was entertained by many medical authors during the first half of the present century and is still held by a few. Thus Cornillac (1886) says: "In the zone which is habitual to it, yellow fever may develop at a given moment without apparent cause. It is born spontaneously at a point of this zone, or at several at a time, and neither the temperature, moisture, barometric pressure, electricity, nor finally

effluvia given off from the soil can explain this sudden invasion." It is true that in localities where the disease is epidemic cases occur which are not directly traceable to importation, but it is also true that in the principal endemic foci of the disease, such as Vera Cruz, Havana, and Rio Janeiro, yellow fever was at one time unknown, and we have reliable historical data fixing the date of its importation. In short, a careful consideration of the historical evidence relating to the disease gives no support to the idea of independent local origin, any more than in the case of smallpox, cholera, or other specific infectious diseases.

But the early history of the disease is involved in obscurity and we are at present unable to determine whether, as maintained by some, it was endemic at certain points on the shores of the Gulf of Mexico at the time of the discovery of the "new world," or whether it was imported to the West Indies from the African coast, as maintained by others. The early historians, Herrera, Oviedo, Rochefort, and others make reference to epidemics among the natives, which occurred prior to the discovery of the Antilles, and to fatal pestilential diseases among the first settlers of these islands, but their accounts are not sufficiently exact to enable us to affirm that the disease referred to by them was yellow fever. The west coast of Africa was discovered and colonized to some extent before the discovery of America, but the first authentic accounts of the prevalence of yellow fever on this coast date back only to the year 1778, over 2 centuries after the first settlements had been established. On the other hand, this very epidemic of 1778, at St. Louis (Senegal), was traced to importation from Sierra Leone, a portion of the African coast, which, according to Hirsch, "appears to be the headquarters of the disease and the starting point of its epidemic inroad into the territories lying to the north and south, as well as into the West African islands."

Rochefort, whose "Histoire naturelle et morale des isles Antilles de l'Amerique" was published in Holland in 1558, says of the West Indies: "The air of all those islands is very temperate and healthy when one is accustomed to it. The *peste* was formerly unknown there as well as in China and other places in the Orient; but some years since the islands were afflicted with malignant fevers, which the physicians considered contagious. The bad air was brought there by some ships which came from the coast of Africa, but at present we hear nothing more of these maladies."

It seems very probable that a pestilential malady which prevailed for a time in these usually healthy islands and then disappeared, was, in fact, yellow fever, and that it was introduced by ships from the west coast of Africa is not at all incredible. Indeed, it almost seems necessary to look for an original endemic focus of the disease outside of the West Indies, for the reason that, in the comparatively few places where it is now endemic, there is historical evidence to show that there was a first importation and a previous period of exemption; while, on the other hand, the conditions upon which endemicity at the present day seems mainly to depend, were formerly unknown—conditions arising from the aggregation of population at seaport cities, as at Havana, Vera Cruz, and Rio Janeiro.

Some authors have attempted to identify the epidemic disease mentioned by Humboldt—called by the natives "Matlazahuatl"—which prevailed in Mexico in 1545, 1576, 1736–'37, and 1761–'62, with yellow fever; but as pointed out by Hirsch, this disease prevailed almost exclusively among the natives of the interior and of the table-land of Mexico, while yellow fever is essentially a disease of the littoral.

Cornillac, a recent French author, who has made a careful study of the sanitary history of the West Indies, as contained in the works of Oviedo, Herrera, Gomara, and other Spanish authors of the sixteenth century, arrives at the conclusion that the pestilential disease from which the settlers in the first Spanish colony at Nueva-Isabella, and at Santo Domingo (1494–1514), are said to have suffered, and which was characterized by a "saffron-yellow" color of the skin, was, in truth, yellow fever. While it appears quite probable that this was so, we can not accept it as demonstrated, as the first authentic accounts of yellow fever in the West Indies date from about the middle of the following century.

In 1635 a French colony was established upon the island of Guadalupe, and shortly after their arrival a pestilential disease appeared among the colonists which, from the account given by Dutertre, a Catholic priest who came to the island 5 years later, is accepted by Hirsch and by Cornillac as having been yellow fever. From Dutertre's account, however, as quoted by Cornillac, it would appear that yellow fever was first imported into the island of Guadalupe in the year 1648, and that the great mortality previously reported was due to other causes. Dutertre says: "During this same year 1648, the *peste*, until then unknown in these islands since they were inhabited by the French, was brought there by some vessels. It commenced at Saint Christophe, and during the 18 months that it lasted carried away nearly one-third of the inhabitants. This epidemic *peste* caused in those who were attacked a violent *mal de tete*, great debility in all the members, and continual vomiting, so that in 3 days it put a man in his tomb. This contagious malady was brought to Guadalupe by a ship from La Rochelle, called *Le Bœuf*."

At Barbadoes the disease may have prevailed for some years prior to its introduction to Guadalupe, but the first authentic account relates to the year 1647. Richard Ligon, who arrived at the island in the month of September of this year, says that the city of Barbadoes was at that time suffering from a scourge which caused great ravages, so that the living scarcely sufficed to bury the dead. According to this author, the cause of the epidemic was unknown; it was uncertain whether it had been imported, or whether it originated from bad food, the use of marsh water, and the intemperance of the colonists. Ligon inclines to attribute it largely to the latter cause, and remarks that not more than one woman died for every ten men. We may safely assume, from the subsequent history of the island of Barbadoes, that the epidemic plague referred to by Ligon was not of local origin, for with a rapidly increasing population this island has enjoyed considerable periods of immunity from yellow fever, and when epidemics have occurred they have, as a rule, been clearly traced to importation. From this time, 1647–'48, the history of yellow fever in the West Indies is a history of epidemic outbreaks at varying intervals at the principal seaport towns, traced sometimes to importation, but more commonly assumed to be of local origin. It was epidemic in Jamaica in 1655, and again in 1671; at Santo Domingo in 1656; at Martinique in 1688 and 1696. In 1699 it prevailed widely as an epidemic in the West Indies, and according to Hinemann made its first appearance at Vera Cruz, the principal seaport on the Gulf coast of Mexico.

Cuba.—I can not attempt to follow here the history of yellow fever in the West Indies generally, but shall give an account of its prevalence in Havana, as this is now an endemic focus of the disease, and the point which is the most dangerous to the UnitedStates, on account of its proximity and the constant commercial intercourse between this city and our own seaports.

The historian Pezuela records the prevalence of a malignant pestilential disease in Havana in 1648, a year in which, as we have seen, yellow fever was epidemic in the islands of Guadalupe and of Barbadoes. He says: "In this year there occurred in Havana a great pest of putrid fevers which remained in the port almost all summer. A large part of the garrison and a larger part of the crew and passengers in the vessels died."

The epidemic continued the following year, and in 1653–'54, according to the author above quoted, "the epidemic was renewed with equal fury;" and in 1655 "in the capital continued to carry away its victims without regard to rivalries and passions." According to Dr. S. E. Chaille, president of the Havana yellow fever commission (1879), from whose report we have quoted the above extracts from Pezuela, there is no historical evidence of the prevalence of yellow fever in Havana for more than a hundred years after the date mentioned. "On the contrary, there are repeated records of the great salubrity of the climate and the absence of epidemic diseases."

It was not until the year 1761 that yellow fever established itself in the previously healthy city. Pezuela gives the following account of its introduction: "Although Havana is situated on the northern boundary of the torrid zone, it was very justly

considered one of the most healthy localities on the island before its invasion in a permanent manner by the *vomito negro*, imported from Vera Cruz in the summer of 1761. * * * In May there came from Vera Cruz, with materials and some prisoners destined for the works on the exterior fortifications of Havana, the men-of-war *Reina* and *America*, which communicated to the neighborhood the epidemic known by the name of 'vomito negro.' At the end of the following June there were stationed in this port nine men-of-war, despatched from Cadiz, and sent to the chief of the squadron, Don Entienne de Hevia; they brought a reinforcement of 2,000 men More than 3,000 persons succumbed to the epidemic on this, the *first appearance* of the vomito."

From this time to the present day the new levies of troops sent from Spain to Cuba have continued to suffer enormous losses from the endemic pestilence. In 1779 there arrived from Spain, then at war with Great Britain, "an army of 3,500 men, which was immediately decimated by the vomito." In 1780, during the month of August, an army of 8,000 men was landed in Havana, which during the two following months suffered a loss of about 2,000 men with the vomito. Pezuela records the fact that in 1794, in the garrison and squadron, there were more than 1,600 victims to the disease.

The endemicity of yellow fever in Havana was fully established by the researches of the commission sent to that city in 1879, by the national board of health. Dr. Chaille, president of this commission, says in his elaborate report, published in 1881: "Since 1761, yellow fever has prevailed certainly in Havana, and probably in other places in Cuba, every year, and the dates of prevalence recorded in our text-books indicate no more than the years of greatest prevalence. The disease prevails in Havana, and in some other places in Cuba, not only every year, but also every month in the year; records in 1837 indicate that at that date the monthly prevalence had become habitual in Havana; the statistics, solely of the military and civil hospitals, prove that during the 408 months, 1856–'79, there was only one single month free from an officially recorded case of yellow fever."

The following tables are from the "Preliminary Report of the Havana Yellow fever Commission:"

Monthly maximum and minimum deaths by yellow fever in Havana during the 10 *years* 1870–'79.

Months.	Minimum.	Maximum.	Months.	Minimum.	Maximum.
January	6	32	July	68	675
February	4	24	August	70	416
March	4	32	September	35	234
April	4	37	October	28	185
May	13	103	November	5	150
June	66	378	December	9	82

In no one of the 10 years, 1870–'79, has there ever been fewer deaths than in the first, nor more than in the second, column. The total deaths by yellow fever for each year were as follows:

Total deaths by yellow fever in Havana.

In 1870	665	In 1875	1,001
In 1871	991	In 1876	1,619
In 1872	515	In 1877	1,374
In 1873	1,244	In 1878	1,559
In 1874	1,425	In 1879, to October 1	1,353

'Matanzas has the reputation of having long suffered annually with yellow fever; the earliest positive date secured by me was reported by Dr. Guiteras, a member of the commission, a native of Matanzas, who was assured by one of the oldest physi-

cians that the city suffered with the disease in 1828, when he came to Matanzas, and it has prevailed every year since." (Chaille, *op. cit.*).

Cienfuegos.—"Yellow fever every year since 1850, except in 1862 and 1874."

Santiago de Cuba.—"Yellow fever prevailed every year, and nearly every month, from 1850 to the present time."

Manzanillo.—"Yellow fever every year since 1866."

Vera Cruz, the principal seaport on the Gulf coast of Mexico, is also the principal endemic focus of yellow fever upon this coast. According to Hinemann, the first epidemic occurred in 1699, a year in which yellow fever was widely prevalent in the West Indies, and in which it prevailed for the first time as an epidemic in the city of Philadelphia.

The following table, which I copy from a paper by Dr. Zacarias R. Molina, a medical officer of the Mexican Army who has for a number of years been on duty in the military hospital at Vera Cruz shows the continued prevalence of the disease in that city during a period of nearly 16 years:

Mortality from yellow fever in the city of Vera Cruz from July, 1867, to December, 1881.

Months.	1867.	1868.	1869.	1870.	1871.	1872.	1873.	1874.	1875.	1876.	1877.	1878.	1879	1880.	1881
January	.....	7	.....	.....	3	2	1	1	7	.....	.....	16	6	2	28
February	.....	6	.....	.....	.....	2	.....	2	2	1	1	5	4	.....	22
March	.....	7	.....	.....	1	4	.....	.....	4	1	.....	.....	2	1	29
April	.....	31	2	.....	6	5	3	.....	11	.....	.....	1	1	1	29
May	.....	42	.....	.....	19	14	1	2	29	.....	4	7	1	.....	94
June	.....	16	.....	.....	113	45	19	3	93	2	7	58	1	.....	233
July	8	28	1	.....	71	53	59	11	118	4	54	114	2	1	132
August	29	21	1	.....	17	39	59	24	105	7	144	110	1	3	39
September	36	21	.....	.....	10	29	74	7	41	9	164	62	3	9	22
October	17	9	1	3	15	11	20	12	13	6	77	45	.....	42	25
November	11	2	2	5	2	.....	10	11	2	1	50	24	.....	92	18
December	8	3	.....	2	4	6	7	6	.....	3	27	7	.....	98	4
Total	109	193	7	10	271	210	223	79	425	34	528	444	21	249	675

There is no evidence of continued prevalence at other towns upon the Mexican coast, but epidemics, which have usually been traced to importation from Vera Cruz, have occurred at *Matamoras* (1858, 1863, 1867); at *Tampico* (1821, 1836, 1845, 1847, 1853, 1864); at *Tuxpan* (1863, 1875, 1877); at *Campeche* (1865, 1877); and at *Manzanillo* (1868).

The Gulf coast of South America, and especially the English and French settlements in Guiana, have been frequently visited by epidemics of yellow fever, and it is probable that the disease is endemic at one or more points upon this coast. Its epidemic prevalence is recorded for the following years at *Demerara*: 1793–96, 1800, 1803, 1818, 1819, 1820, 1821, 1825, 1827, 1828, 1831, 1837–'39, 1841–'45, 1851–'53, 1861–'66 (Hirsch).

In Venezuela the disease has prevailed at the capital, *Caracas*, and the neighboring seaport, *La Guayra*, in the years 1693, 1696, 1793, 1797, 1802 and 1869.

In Central America epidemics have occurred at all of the principal seaports: *Panama* 1740, 1858, 1859, 1867; *Portobello*, 1726, 1729, 1740, 1793, 1860, 1866, 1867; *Belize*, 1860; *Nicaragua*, 1868.

Brazil.—The Portuguese author, El Hastio da Rocha Pitti, has given an account, in his "History of Portuguese America," published in Lisbon in the year 1730, of an epidemic malady which prevailed in Pernambuco in the year 1686, which very probably was yellow fever. This author says (Book VII., p. 427 *et seq.*): "In the year 1686, commenced in Pernambuco that terrible plague (contagious disease, *Bicha*) which must be attributed to the sins of the population of these provinces, corrupted by the

vices into which they were enticed by the wealth and freedom of Brazil. Many causes are alleged, the most worthy of attention being the arrival of some barrels of meat which returned from the island of Sao Thome (St. Thomas). These were opened by a cooper, who shortly afterward fell dead. Soon after several persons of his family, to whom he had communicated the disease, also died. The epidemic spread to such an extent among the inhabitants of Recipe (Pernambuco) that the mortality exceeded 2,000, which was very large in proportion to the population. Thence the disease extended to Olinda and its vicinity, and very few were the persons who escaped it, such were its virulence and intensity." The account given by the historian of the clinical features of this pestilential disease is, of course, very imperfect, but it seems to justify the belief that the disease was really yellow fever.

The highest medical authorities in Brazil agree that yellow fever was not endemic in the principal seaports of the Empire prior to the year 1849, when it was introduced to the city of Bahia by the North American brig *Brazil*, which sailed from New Orleans, where yellow fever was prevailing, and touched at Havana. Two of the crew of this brig died of yellow fever during her voyage from the latter port to Bahia. Soon after her arrival the disease made its appearance among those who had communicated with the ship, and later on other vessels in the harbor. The first case occurred a few days after the arrival of this brig (November 3). A part of her cargo is said to have consisted of little barrels of beef which had become putrid. From Bahia the disease was carried to Rio Janeiro, where during the epidemic season of 1850 it caused a mortality of 4,160.

According to Professor Barata, of the faculty of medicine of Rio Janeiro, yellow fever continued to prevail in Brazil until the year 1861, when it disappeared for 8 years, to reappear in 1869–'70, as the result of a fresh importation. The Italian ship *Creolla del Plata*, which had touched at St. Iago, where yellow fever was prevailing, is named as the vessel which introduced the disease on this occasion.

The mortality from the disease under consideration in the city of Rio, from the time of its introduction in 1850 to a recent date, is shown by the following table:

	Mortality.		Mortality.
1850	4,160	1869	274
1851	475	1870	1,117
1852	1,943	1871	8
1853	853	1872	102
1854	21	1873	3,659
1855	0	1874	829
1856	0	1875	1,292
1857	1,425	1876	3,317
1858	800	1877	282
1859	500	1878	1,174
1860	1,249	1879	974
1861	247	1880	1,433
1862	12	1881	219
1863	0	1882	95
1864	0	1883	1,336
1865	0	1884	618
1866	0	1885	278
1867	0	1886	1,397
1868	0		

In 1855 yellow fever is said, by Hirsch, to have prevailed extensively in Brazil, although this was not an epidemic year in Rio Janeiro. The following year it extended along the Amazon far into the interior of the country. The years of greatest epidemic prevalence since that date have been 1859–'60, 1862, 1869–'70, 1872–'73, 1875–'77 (Hirsch).

From Brazilian ports the disease has occasionally been introduced to the cities at the mouth of the Rio de la Plata, and has there caused great loss of life. The first

epidemic at *Montevideo* was in 1857, and it was again introduced into this city from Pernambuco in 1872. It prevailed in the city of *Buenos Ayres* in 1858 and in 1870.

Yellow fever is said to have been conveyed to the Pacific coast of South America by a party of German emigrants, who landed at Callao, *Peru*, in 1854. The disease spread from this port to the capital, and in the course of the next 2 or 3 years to the principal towns upon the Peruvian coast, where it continued to prevail up to the year 1869.

Chili, up to the present time, has remained exempt from the disease (Hirsch).

Upon the *west coast of Africa* the headquarters of yellow fever is that portion of the coast which belongs to the province of *Sierra Leone*, and epidemics at other points upon the African coast have frequently been traced to this locality. It seems very doubtful, however, whether, as some authors suppose, this is really the original source of the disease. The French authors, Berenger-Feraud and Bourru, both call attention to the fact that we have no account of the disease prior to the year 1778, although the African coast was discovered and colonized long before the discovery of the West Indies; and that, on the other hand, the early settlers in these islands suffered from a pestilential malady which very probably was yellow fever.

At *St. Louis* (Senegal) an epidemic occurred in 1778 and this is the first outbreak of the disease of which we have any reliable information in this portion of the world. The disease in this instance is said by Schotte to have been imported from *Sierra Leone*, where epidemics are recorded to have occurred during the present century in 1816, 1823, 1825, 1829–'30, 1837–'39, 1845–'47, 1859, 1862, 1864, 1865–'66, 1868, 1878 (?) (Hirsch). Frequent epidemics have also occurred at *Senegambia*, and the disease has prevailed upon the *Gold Coast* (1852, 1857, 1862), the *Congo Coast* (1816, 1860, 1862, 1865), at the *Cape Verde Islands* (1845, 1862, 1868), and the *Canary Islands* (1701, 1771, 1810, 1846, 1862).

In EUROPE the ravages of yellow fever have been restricted mainly to the Iberian peninsula. This is due, no doubt, to the frequent intercourse between Spain and Portugal and the West Indian ports, in which the disease is most prevalent, and to the fact that the summer temperature of these countries is favorable for the epidemic extension of the disease, whereas the more northern portions of Europe are practically outside of the yellow fever zone.

The first epidemic in *Spain* occurred in the year 1700, at Cadiz. This city also suffered in 1730–'31, 1733–'34, 1764, 1780, 1800, 1804, 1810, 1819–'21. The epidemics of 1800, 1810, and 1819 were not limited to the city of Cadiz, but the disease extended to the interior and caused a considerable loss of life in the provinces of Granada and Adalusia, and also in some of the towns of Murcia and Catalonia—especially in Barcelona, from which city the disease was conveyed to the island of Majorca during the last epidemic. No wide-spread epidemic has occurred in Spain since 1821, but local outbreaks, as a result of importation from the West Indies, have occurred in Gibraltar (1828), Barcelona (1870), and Madrid (1878).

The first epidemic at *Lisbon* was in 1723, a second was inaugurated in 1856, and during the following year developed into a devastating scourge which extended to the towns of Belem, Olivæs, and Almada.

In *Italy* yellow fever has only once effected a temporary lodgement, at Leghorn, in 1804, where it was imported from Spain.

Ships with yellow fever on board have occasionally arrived at English and French ports, but local conditions have apparently not been favorable to an extension of the disease, except to a limited extent at Brest, in 1856, at St. Nazaire, in 1861, and at Swansea (Wales), in 1864.

BAHAMA ISLANDS.

Bahama Islands.—Yellow fever prevailed as an epidemic at Nassau in 1861, 1862, 1863, and in 1869.

According to Hirsch yellow fever prevailed, to a limited extent, at Halifax (latitude 44° 26′) in 1861, and at Quebec (latitude 46° 50′) in 1805.

PREVALENCE OF YELLOW FEVER IN THE UNITED STATES.

New Hampshire.—Portsmouth is the most northern point in the United States which has suffered an epidemic of yellow fever. In 1798, and again in 1802, during which year the disease was epidemic in New York and in Philadelphia, it was also epidemic in this city.

Massachusetts.—In 1693 an English expedition sailed from Boston for the purpose of taking from the French the Island of Martinique. The expedition failed in its object and returned to Boston on June 17 with yellow fever on board the vessels of the fleet. Hutchinson, in his "History of Massachusetts Bay," says the mortality among the sailors had been 1,300 out of a total strength of 2,100, and that out of the same number of soldiers the loss was 1,800. He states that the disease spread from the fleet to the town, and that many families left town and resided in the country until the infection had ceased. This is the first authentic account of the occurrence of yellow fever within the present limits of the United States. In 1796 the disease prevailed to a limited extent in Boston and in Newburyport. In 1798 it prevailed as an epidemic in Boston, where the mortality was 200; and in 1802 60 fatal cases occurred in the same city. Some cases also occurred in the years 1800, 1819, and 1858.

Rhode Island.—The city of Providence was several times visited by yellow fever during the latter part of the eighteenth and the beginning of the present century—1794, 1795 (mortality 45), 1797 (mortality 45), 1800, 1805. The disease prevailed at Newport in 1798, and at Bristol in 1797.

Connecticut.—The disease prevailed at New London in 1795, and again in 1798, when the mortality was 81. Hirsch records the occurrence of the disease at New Haven in 1743, 1794, and 1805; at Middletown in 1820; at Chatham in 1796, and at Hartford in 1799.

New York.—Epidemics of greater or less extent have occurred in New York City and its immediate vicinity in 1693, 1702, 1743 (mortality 217), 1745, 1762, 1791, 1794, 1795 (mortality 730), 1798 (mortality 2,080), 1799 (mortality 76), 1800, 1801, 1803 (mortality 700), 1805 (mortality 340), 1809, 1819, 1822 (mortality 230), 1848, 1853, 1854, 1856, 1870 (mortality 49).

New Jersey.—Hirsch records the following local epidemics: Bridgetown, 1798; Chews, 1798; Woodbury, 1798; Perth Amboy, 1811.

Pennsylvania.—According to La Roche, "the earliest onset of the disease occurred in 1699, when Philadelphia, then but seventeen years of age, was little more in point of extent than an ordinary country town." There are no medical accounts of this epidemic, but there is no doubt as to the nature of the disease, which caused a mortality of 220 in the new city, estimated to have contained less than 4,000 inhabitants. The next epidemic in Philadelphia occurred in 1741, when the mortality was 250. Subsequent epidemics occurred in 1747, 1762, 1793 (mortality 4,041), 1794, 1797 (mortality 1,300), 1798 (mortality 3,500), 1799 (mortality 1,000), 1802 (mortality 307), 1803 (mortality 195), 1805 (mortality 400), 1819, 1820 (mortality 83), 1853 (mortality 128), 1870 (mortality 18).

Delaware.—In the epidemic of 1798 the city of Wilmington suffered a loss of 250.

Maryland.—Epidemics, for the most part of limited extent, have occurred in Baltimore in the years 1783, 1794, 1797, 1798, 1799, 1800, 1802, 1819, 1820, 1821, 1822, 1868, 1876.

Virginia.—At Norfolk epidemics are recorded as follows: 1737, 1741, 1794, 1795, 1797, 1799, 1800 (mortality 250), 1801, 1821, 1826, 1855 (mortality 1,807). An epidemic occurred at Petersburgh in 1798, and at Alexandria in 1803. At Portsmouth the disease prevailed in 1852, 1854, and 1855 (mortality 1,000).

North Carolina.—Wilmington, 1796, 1800, 1821, 1862 (mortality 446); Newbern, 1799, 1864 (mortality 700); Beaufort, 1854, 1864 (mortality 68), 1871; Washington, 1800; Smithville, 1862.

South Carolina.—The first epidemic of which we have any account in Charleston

occurred in 1693; from this time epidemics have been numerous, and during the first half of the present century the physicians of Charleston generally considered the disease endemic in that city. That it was not seems to be demonstrated by the immunity enjoyed since 1871, an immunity which is probably due to the diminished commerce with infected ports in the West Indies, and to a more efficient quarantine service, since the fact has been recognized that the disease is not endemic.

The prevalence of yellow fever in Charleston during the present century is shown in the following table; recorded epidemics, prior to the year 1800, are as follows: 1693, 1699, 1700, 1703, 1728, 1732, 1734, 1739, 1745, 1748, 1753, 1755, 1761, 1762, 1768, 1770, 1792, 1794, 1795, 1796, 1797, 1798, 1799 (mortality 239). An epidemic occurred among the troops stationed at Hilton Head in 1862; Port Royal, 1877 (mortality 25).

Mortality from Yellow Fever in Charleston, S. C.; Pensacola, Fla.; Mobile, Ala.; New Orleans, La.; and Galveston, Tex., during the present century.

Year.	Charleston.	Pensacola.	Mobile.	New Orleans.	Galveston.	Year.	Charleston.	Pensacola.	Mobile.	New Orleans.	Galveston.
1800	184	.....	.....	(*)	.....	1841	(*)	(*)	(*)	594	.....
1801	.....	.....	.....	(*)	.....	1842	.....	(*)	60	211	.....
1802	96	.....	.....	(*)	.....	1843	1	(*)	240	487	.....
1803	(*)	.....	.....	.....	.....	1844	.....	(*)	(*)	148	400
1804	148	.....	.....	(*)	.....	1845	.....	(*)	.....	2	.....
1805	(*)	.....	.....	.....	.....	1846	.....	(*)	.....	160	.....
1806	.....	.....	.....	.....	.....	1847	.....	(*)	76	2,259	200
1807	162	.....	.....	.....	.....	1848	.....	(*)	75	850	.....
1808	.....	.....	.....	.....	.....	1849	125	.....	50	737	.....
1809	.....	.....	.....	(*)	.....	1850	.....	.....	.....	102	.....
1810	.....	.....	.....	.....	.....	1851	.....	.....	(*)	16	.....
1811	.....	(*)	.....	(*)	.....	1852	310	.....	.....	415	.....
1812	(*)	.....	.....	(*)	.....	1853	.....	(*)	115	7,970	536
1813	.....	.....	.....	.....	.....	1854	627	(*)	(*)	2,423	404
1814	.....	.....	.....	.....	.....	1855	.....	.....	.....	2,670	.....
1815	.....	.....	.....	.....	.....	1856	211	.....	.....	74	.....
1816	.....	.....	.....	.....	.....	1857	13	.....	.....	199	.....
1817	272	.....	.....	800	.....	1858	717	(*)	(*)	3,889	344
1818	.....	.....	.....	115	.....	1859	.....	.....	.....	.....	182
1819	177	.....	274	2,190	.....	1860	.....	.....	.....	.....	.....
1820	.....	.....	.....	(*)	.....	1861	.....	.....	.....	.....	.....
1821	.....	.....	(*)	.....	.....	1862	(*)	(*)	.....	.....	.....
1822	2	257	(*)	239	.....	1863	.....	(*)	.....	.....	.....
1823	.....	.....	.....	1	.....	1864	(*)	.....	.....	.....	259
1824	235	.....	(*)	108	.....	1865	.....	.....	.....	.....	.....
1825	2	(*)	(*)	49	.....	1866	.....	.....	.....	.....	(*)
1826	.....	.....	.....	5	.....	1867	.....	34	(*)	3,093	1,150
1827	64	(*)	(*)	109	.....	1868	.....	.....	.....	.....	.....
1828	26	.....	(*)	130	.....	1869	.....	.....	.....	.....	.....
1829	.....	.....	130	215	.....	1870	.....	.....	(*)	587	.....
1830	30	.....	.....	117	.....	1871	213	.....	.....	55	.....
1831	.....	.....	.....	2	.....	1872	.....	.....	.....	40	.....
1832	.....	.....	.....	18	.....	1873	.....	61	27	225	.....
1833	.....	.....	.....	210	.....	1874	.....	118	.....	.....	.....
1834	49	(*)	.....	95	.....	1875	.....	.....	.....	.....	.....
1835	25	.....	.....	284	.....	1876	.....	.....	.....	.....	.....
1836	.....	.....	.....	5	.....	1877	.....	.....	.....	.....	.....
1837	.....	.....	350	442	.....	1878	.....	.....	90	600	.....
1838	251	.....	.....	17	.....	1879	.....	.....	.....	.....	.....
1839	134	(*)	650	452	250	1880	.....	.....	.....	.....	.....
1840	22	.....	.....	3	.....						

(*) Number of deaths not stated.

Georgia.—At Savannah epidemics are recorded in the years 1800, 1807, 1808, 1817, 1819, 1820, 1827, 1852, 1853, 1854 (mortality 580), 1858, 1876; at St. Mary's in 1808 (mortality 84) and in 1854; at Augusta in 1839 and 1854; at Bainbridge in 1873; Brunswick, 1876.

Florida.—The principal seaport, Pensacola, has suffered frequent epidemics of yellow fever. Those occurring during the present century are included in the table given above. Two epidemics are recorded as occurring prior to the year 1800—1764 and 1765. At St. Augustine epidemics occurred in 1807, 1821, 1838, 1839, and 1841; at Key West in 1823, 1829, 1841, 1862, 1867, 1875, 1878, 1887; at Jacksonville in 1857, 1877, and 1888; at Fernandina in 1877 (mortality 498); at Tampa in 1839, 1853, 1871, 1887.

Alabama.—The recorded epidemics in Mobile, prior to the year 1800, were in 1705, 1765, and 1766; subsequent epidemics are included in the table. Montgomery, 1853 (mortality 35), 1854 (mortality 45), 1855 (mortality 30), 1873 (mortality 102); Selma, 1853 (mortality 32); Florence, 1878.

Mississippi.—The town of Biloxi, on the Gulf, has suffered from epidemics as follows: 1702, 1839, 1847, 1853, 1858, 1878, 1884; Pascagoula, 1847, 1853, 1875, 1878; Port Gibson, 1878; Shieldsborough, 1820, 1829, 1839; Port Adams, 1839, 1853; Grand Gulf, 1853; Natchez, on the Mississippi River, 1817, 1819 (mortality 180), 1823 (mortality 312), 1825 (mortality 150), 1827, 1829 (mortality 90), 1837 (mortality 280), 1839 (mortality 235), 1848, 1853, 1855, 1858; Vicksburg, 1839, 1841, 1847, 1853, 1855, 1858, 1871, 1873, 1878 (mortality 872); Jackson, 1853, 1854, 1878 (mortality 86); Holly Springs, 1878 (mortality 309); Greenville, 1878 (mortality 301); Grenada, 1878 (mortality 326); Canton, 1878 (mortality 180). Our record does not include numerous smaller places which suffered during the epidemic of 1878.

Louisiana.—The first recorded epidemic in New Orleans was in the year 1769; other outbreaks prior to the present century were in 1791, 1793, 1794, 1795, 1796, 1797, 1799. The prevalence of the disease in this city subsequent to the year 1800 is given in the table. Baton Rouge, 1817, 1819, 1822, 1827, 1829, 1837, 1843, 1847, 1853, 1858, 1878 (mortality 193); Opelousas, 1837, 1839, 1842, 1853; St. Francisville, 1811, 1817, 1819, 1823, 1827, 1829, 1839, 1843, 1846, 1848, 1853; Shreveport, 1853, 1873 (mortality, 759); Port Hudson, 1839, 1841, 1843, 1853, 1878; Thibodeaux, 1846, 1853, 1854, 1878; Washington, 1837, 1839, 1853, 1854, 1867; Morgan City, 1878 (mortality 109). Numerous smaller places during the epidemics of 1873 and of 1878.

Texas.—The epidemics at Galveston are included in our table. Houston, 1839, 1844, 1847, 1848, 1853, 1854, 1858, 1859, 1864, 1867, 1870; Huntsville, 1867 (mortality 130); Hempstead, 1867 (mortality 151); Indianola, 1852, 1853, 1858, 1859, 1862, 1867 (mortality 80); La Grange, 1867, (mortality 200); Matagorda, 1862 (mortality 120); Navazota, 1867 (mortality 154); Rio Grande City, 1867 (mortality 150); Victoria, 1867 (mortality 200); Brenham, 1867 (mortality 120); Calvert, 1867 (mortality 250); Chapel Hill, 1867 (mortality 123); Columbia, 1867 (mortality 132); Brownsville, 1853, 1858, 1862, 1882.

Tennessee.—Memphis, 1828, 1853, 1855, 1867, 1873 (mortality 1,244), 1878 (mortality 5,000), 1879 (mortality 485); Chattanooga, 1878 (mortality 135); Brownsville, 1878 (mortality 212); numerous smaller towns in 1878.

Arkansas.—Columbia, 1853; Fort Smith, 1823; Little Rock, 1873; Napoleon, 1853.

Kentucky.—Bowling Green, 1878; Hickman, 1878 (mortality 153); Louisville, 1878 (mortality 64).

Ohio.—Cincinnati, 1871, 1873, 1878 (mortality 17); Gallipolis, 1796, 1878 (mortality 18).

Illinois.—Cairo, 1873 (mortality 17), 1878 (mortality 51).

Missouri.—St. Louis, 1854, 1855, 1878 (mortality 16); New Design, 1797 (mortality 57).

GREAT EPIDEMICS IN THE UNITED STATES.

1793.—The city of Philadelphia, after enjoying an immunity from yellow fever for 31 years, suffered in 1793 a devastating epidemic. This epidemic, no doubt, resulted from importation, although a clear history of its introduction was not made out at

the time, and the leading physicians of the city were inclined to attribute it to local origin, as a result of unsanitary conditions in connection with an unusually high temperature. La Roche says: "Dr. Rush and others laid great stress on a quantity of damaged coffee which was exposed during the latter part of July, in a place (on a wharf and in the adjoining dock) and under circumstances which favored decomposition. Its smell was highly putrid and offensive, insomuch that the inhabitants of the houses in Water and Front streets, who were near to it, were obliged in the hottest weather to exclude it by shutting the doors and windows. Even persons who only walked along those streets complained of intolerable fetor, which, upon inquiry, was constantly traced to the putrid coffee."

It appears probable that this "putrid coffee" was indeed the nidus in which the deadly exotic germ first developed which gave rise to this fatal epidemic. Whether the coffee was infected at the port of shipment, or whether it was transported in an infected vessel, we can not now determine; but that the outbreak of yellow fever in Philadelphia was due to the fact that the coffee was imported from a region where yellow fever was prevailing, or in an infected ship, rather than to the fact that it was putrid, can not be doubted, in view of the subsequent history of yellow fever epidemics in the United States.

As usual, the early cases were not recognized as yellow fever. Dr. Rush says: "The report of a malignant and fatal fever being in town spread in every direction, but it did not gain universal credit. Some of those physicians who had not seen patients in it denied that any such fever existed, and asserted (though its mortality was not denied) that it was nothing but the common annual remittent of the city. Many of the citizens joined the physicians in endeavoring to discredit the account I had given of this fever, and, for a while, it was treated with ridicule or contempt. Indignation in some instances was exerted against me." History has repeated itself, in this particular, many times in subsequent epidemics. The early cases, even in cities like New Orleans, where the physicians are well acquainted with the disease, are frequently called by some other name—"bilious fever," "pernicious fever," "malarial fever," etc.—and the physician who first ventures to name the prevailing disease "yellow fever" is treated with ridicule or with indignation.

It was not until the middle of August that a rapid succession of fatal cases convinced the physicians of the city that the fatal West Indian pestilence was again present in Philadelphia.

The presence of the disease was officially recognized on the 22d of August, when the mayor of the city gave orders for the cleaning of the streets and general purification of the city. The disease continued to extend until early in October, when it reached its height. It did not cease entirely until about the 8th of November. During this short season of prevalence it caused an enormous mortality, distributed as follows: "August, 325; September, 1,442; October, 1,976; November, 118." (La Roche).

The population of the city at this time is estimated to have been a little more than 40,000, which gives a mortality of 10 per cent. of the total population (total mortality 4,040). As more than 12,000 of the inhabitants fled from the city, the proportion of those who were attacked is very great. La Roche estimates the total number of cases at 11,000.

1797.—The epidemic of this year in the city of Philadelphia was less extended and less fatal. The whole number of deaths is estimated to have been about 1,300. The disease, as usual, commenced in the vicinity of the wharves (about the end of July). Unsanitary conditions, described by physicians who were witnesses of the epidemic, furnished the favorable local nidus for the exotic germ, which, according to a report of the College of Physicians of Philadelphia made in response to a request from the governor, was imported by two vessels, one from Havana and the other from Port au Prince. In this report the College of Physicians, contrary to the prevailing popular opinion, and that of many prominent physicians, took the ground that the unsanitary

local conditions were simply secondary or accessory causes, and recommended "a more stringent system of quarantine regulations, as the most effectual means of preventing the recurrence of the disease" (La Roche).

1798.—The epidemic of 1797 was followed the next year by a still greater one, which was not confined to the city of Philadelphia alone. The disease prevailed also in Boston (mortality 200), in Portsmouth, N. H. (mortality 100), in Newport, R. I. (mortality 2), in New London, Conn. (mortality 81), in New York (mortality 2,080), in Wilmington, Del. (mortality 250), and in Charleston, S. C. The mortality in Philadelphia was 3,645, distributed as follows: August, 626; September, 2,004; October, 943; November (from the 1st to the 5th), 72. The mortality, in proportion to the number of cases, in the city of Philadelphia was enormous, being, according to La Roche, about as 1 to 1.27 of those attacked, or nearly 80 per cent. This is accounted for partly by the fact that the better class of the community left the city as soon as possible after the outbreak of the disease, and the cases which occurred were consequently among the poorer classes, who inhabited the worst portions of the city. The prevailing ideas as to the treatment of fevers by depleting measures, were doubtless responsible to some extent for the excessive mortality. "The College of Physicians, faithful to the theory so long entertained by it in relation to the cause of the disease, assigned to the epidemic this year, as it had done to those of preceding seasons, a foreign origin" (La Roche).

1802.—An epidemic of smaller proportions prevailed in the year 1802, causing a mortality in Boston of 60, in Philadelphia of 307, in Wilmington of 86, in Charleston of 96. The disease also prevailed "extensively" in Baltimore, but no record of mortality is given. The prevalence of the disease at the seaports mentioned, especially before the time of railroad communication, is not to be ascribed to an extension from one to the others, or to "an epidemic constitution of the atmosphere;" but it doubtless occurred, for the most part, as a result of independent importation from the usual source of the disease, the West Indies. Thus we find that in 1802, while Boston and Philadelphia suffered epidemics, New York, lying between the two infected points, was free from the disease (two cases only are reported).

1853.—Passing over the minor epidemics, for the most part limited to a single city, or, by coincidence merely, to two or more distant sea-ports, we come to the epidemic of 1853, which extended through portions of the States of Florida, Alabama, Louisiana, Mississippi, Arkansas, and Texas. The towns which suffered in *Florida* were Pensacola, Milton, and Tampa. In *Alabama:* Mobile (mortality 115), Cahawba, Citronelle, Demopolis, Fulton, Hollywood, Montgomery (mortality 35), Selma (mortality 32), were the principal towns visited by the scourge. In *Louisiana* the disease prevailed at New Orleans, with a mortality of 7,970; at Alexandria, Algiers, Bay St. Louis, Bayou Sara, Centreville, Clinton, Coultierville, Franklin, Opelousas, Pattersonville, Plaquemine, Shreveport, Thibodeaux, Trenton, Washington, and various smaller places. In *Mississippi:* Biloxi, Brandon, Clinton, Grand Gulf, Greenwood, Jackson, Natchez, Pascagoula, Pass Christian, Port Gibson, Washington, Woodville, Yazoo. In *Arkansas:* Columbia, Grand Lake, Napoleon. In *Texas:* Brownsville, Cypress City, Galveston, Hockley, Houston, Indianola, Liverpool, Richmond, Saluria.

1867.—The epidemic of this year was widely extended in the State of Texas. The first recognized case in New Orleans occurred on the 10th of June. The total mortality in this city was 3,093. Other towns visited in Louisiana were New Iberia and Opelousas. In *Texas* the first cases occurred at Galveston on the 26th of June, and the total mortality in this city was 1,150. Other places visited by the epidemic were Alleyton, Anderson, Austin, Bastrop, Brenham, Calvert (mortality 250), Chapel Hill (mortality 123), Corpus Christi, Danville, Goliad, Hempstead (mortality 151), Huntsville (mortality 130), Independence, Indianola (mortality 80), La Grange (mortality 200), Liberty, Millican, Navazota (mortality 154), Oldtown, Port Lavacca, Rio Grande City (mortality 150), Victory (mortality 200).

1873.—Florida, Alabama, Mississippi, Louisiana, and Texas again suffered from an epidemic of yellow fever in the year 1873. At Pensacola, Fla., the first recorded

case occurred August 6, and the total mortality was 61. In *Alabama* the disease appeared at Mobile on the 21st of August, and the total mortality was but 27; Montgomery suffered a loss of 102. In *Louisiana* the mortality in the city of New Orleans was only 225, although the epidemic had its origin in this city. It was imported by the Spanish bark *Valparaiso,* which sailed from Havana June 15 in ballast, arrived at New Orleans quarantine station June 24, was detained 2 days, and came to the city June 26. The first case was the mate of this vessel, who was taken sick on board July 4, while she was lying at the wharf. But for the sickness and death of the mate of the *Valparaiso* the origin of this epidemic would have remained obscure, and the believers in the local origin of the disease would have had a strong case, for no other cases of the disease occurred on the *Valparaiso.* This is explained by the fact that the crew consisted of acclimated Spaniards, and the mate seems to have been the only susceptible person on board who could serve as a test of the infection of the vessel at her port of departure. From New Orleans the disease was carried to Memphis by the river steamer *Bee.* It caused a mortality in this city of 2,000. River steamers from New Orleans also carried the disease to Shreveport, La., where the mortality was 759. From Shreveport a refugee fled to the town of Calvert, Tex., where he was taken sick and died. An epidemic followed with a total mortality of 125. The disease was also introduced by refugees to the town of Marshall, Tex., where 36 deaths occurred. The epidemic of this year at Pensacola, Fla., was due to an independent importation by the ship *Golden Dream,* and Montgomery, Ala., became infected through refugees from Pensacola.

1878.—The last and most extended epidemic of yellow fever in the United States is that of 1878, which invaded 132 towns and caused a mortality of 15,934 out of a total number of cases exceeding 74,000.

The origin of this epidemic was traced by the president of the Louisiana State Board of Health (Chopin) to the steamer *Emily B. Souder,* which arrived from Havana, May 23, and was moored at the foot of Calliope street, New Orleans. Dr. Chopin says: "The first cases of yellow fever in New Orleans in 1878 were undoubtedly two of the officers of the above steamship, namely, Clarke, the purser, and Elliott, one of the engineers." Infected centers were developed in the vicinity of the houses in which these men were sick, but not until after an interval of several weeks, during which, probably owing to unfavorable conditions as to temperature, the "germs" remained dormant, or at least multiplied so slowly as not to cause an outbreak of the disease.

Fortunately this great epidemic has been carefully studied by a "board of experts, authorized by Congress," and we have a very complete history of its geographical extension, and of the deadly results which marked its course. The following data are from the report of this "board of experts."

Louisiana.—New Orleans mortality, 4,600; Allamands Station, 17; Baton Rouge, 193; Bayou Cypre, 7; Berwick City, 7; Buras Settlement, 3; Clinton, 15; Delhi, 34; Delta, 47; Donaldsonville, 71; Gretna, 53; Hammond, 5; Henderson, 18; Houma, 6; Jesuits Bend, 2; Labadieville, 24; La Fourche, 26; Lagonda and other plantations, 42; Morgan City, 100; Napoleonville, 8; Paincourtville, 15; Pattersonville, 47; Pilot Town, 17; Plaquemine, 125; Ponchatoula, 3; Port Eads, 13; Port Hudson, 11; St. Bernard Parish, 7; Tangipahoa, 50; Thibodeaux, 65; Teche country plantations, 81.

Tennessee.—Bartlett, 9; Brownsville, 212; Chattanooga, 135; Colliersville, 56; Germantown, 35; Grand Junction, 74; La Grange, 37; Martin, 40; Mason, 24; Memphis, 5,000; Milan, 12; Moscow, 35; Nashville, 6 (all imported cases); Paris and suburbs, 23; Somerville, 57; White Station, 50; Williston, 11.

Alabama.—Decatur, 44; Florence, 50; Huntsville, 12; Leighton, 1; Mobile, 90; Stevenson, 6; Town Creek, 4; Tuscaloosa, 2; Tuscumbia, 31.

Mississippi.—Bay St. Louis, 82; Benton, 1; Biloxi, 45; Bolton, 34; Bovina, 7; Brown's plantations, 4; Canton, 180; Vicinity of Canton, 47; Dry Grove, 41; Friar's Point, 7; Gainsville, 2; Goodrich Landing, 12; Greenville, 301; Grenada and vicinity, 343; Horn Lake, 2; Handsborough, 16; Hernando, 80; Holly Springs, 309; Iuka, 3;

Jackson, 86; Lake, 86; Lebanon, 10; Livingston, 10; McComb City, 21; Meridian, 91; Mississippi City, 15; Ocean Springs, 30; Osyka, 45; Pass Christian, 23; Pearlington, 24; Port Gibson, 115; country about Port Gibson, 150; Refuge Landing, 11; Rocky Springs, 38; Scranton, 20; Stoneville, 15; Spring Hill, 6; Sulphur Springs, 5; Senatobia, 7; Terrene, 4; Vicksburg, 872; vicinity of Vicksburg, 300; Water Valley, 64; Winona, 3; Winterville and vicinity, 26; Yazoo City, 9.

Kentucky.—Bowling Green, 19; Hickman, 153; Louisville, 64 (mostly refugees).

Ohio.—Cincinnati, 17 (refugees); Gallipolis, 18.

Illinois.—Cairo, 51.

Missouri.—St. Louis, 16; quarantine (near St. Louis), 42.

ETIOLOGY.

The preceding historical record shows that in the United States, as elsewhere, yellow fever has prevailed more frequently in seaports than in inland towns, and that, when epidemics prevail in the interior, their origin can commonly be traced to the nearest seaport, or to intermediate towns in communication with it. Towns upon or near the coast which have no commerce are no more subject to invasion by yellow fever than are interior towns, unless it be by reason of their proximity to a seaport. Moreover, the frequency of epidemics in our southern seaports, before the era of efficient quarantine administration, bears a direct ratio to their commercial importance, and especially to their commercial intercourse with Havana or other endemic foci of the disease. Thus New Orleans suffered epidemics of greater or less magnitude in 48 out of the first 60 years of the present century. During the same period (1800–1860) Charleston suffered 28 epidemics; Mobile, 22; Pensacola, 17; Savannah, 9; Galveston, 7. That local conditions are favorable for the development of an epidemic at many of our interior towns, especially those located on great rivers near the sea level in the Southern States, is amply proved by the epidemic of 1878. That yellow fever does not occur at these towns, except as a result of the introduction of infected persons or articles, is beyond question. So, too, in seaport cities there is no reason for believing that any radical change has occurred in local conditions during the past 28 years; yet, during this time New Orleans has only suffered 6 epidemics, while during a corresponding period (28 years) prior to 1860 there were 22 years of epidemic prevalence of the disease. A similar comparison for Charleston shows 14 years of epidemic prevalence prior to 1860, and only one since.

Up to the year 1860 there were many advocates of the *local origin* of the disease in these seaports, and the disease was considered endemic by many physicians, both in Charleston and in New Orleans. But to-day scarcely any one questions the fact that the disease, notwithstanding its frequent prevalence, was due to importation, and that it is nowhere endemic within the boundaries of the United States. It is not improbable, however, that in certain instances the "germs" of the disease have survived the winter season, and that "sporadic cases" and epidemics have occurred as a result of importation dating back one or more years. It is claimed that the epidemic of 1879, in the city of Memphis, was not due to a new importation, but resulted from the hibernation of germs in houses infected in 1878. Dr. Thornton, President of the Memphis Board of Health during these epidemics, says: "The disease appeared in houses in the suburbs, which were infected last year;" and states further that the first case reported to the health office occurred on the 8th of July, at which date the disease was not prevailing in any part of the United States. In New Orleans, in epidemic years, cases have sometimes continued to occur during the greater part of the month of December, and in Tampa and Plant City, Fla., where yellow fever was epidemic in the summer of 1887, cases are said to have occurred at intervals throughout the winter. Admitting, then, the probability that the recurrence of the disease in our Southern seaports has sometimes been due to the preservation of infectious material in an active state throughout the winter, we must insist, never-

theless, that there is no satisfactory evidence of the *de novo* origin of the disease from local causes, either in our own country or elsewhere; and that, wherever its original habitat may have been, the prevalence of the disease within the period to which our authentic historical records relate has been due to the importation of cases, or of infected material, from a previously infected place. In other words, the disease is due to a specific infectious agent.

As to the nature of the *specific cause* of the disease there can scarcely be two opinions. The present state of science justifies the belief that it is a living microörganism; and facts relating to the origin and extension of epidemics show that, as in cholera and in typhoid fever, this microörganism is capable of development outside of the human body under favorable conditions which will be discussed hereafter. Unfortunately, the present state of science does not enable us to give an account of the deadly *microbe* which we assume to be the cause of the disease under consideration. We know to-day the morphological and physiological characters and the habitat within the body of an infected individual of the specfic cause of cholera, of typhoid fever, and of relapsing fever, but the researches made up to the present time have failed to demonstrate the "germ" of yellow fever.

SUSCEPTIBILITY.

Individuals of every race and of all ages, who are exposed to the yellow-fever poison for the first time during the epidemic prevalence of the disease, are subject to be attacked. But there is a wide difference in the degree of this susceptibility among races, and among individuals of the same race.

RACE.

It has been asserted that the negro race has a congenital immunity from yellow fever, but this is a mistake. The susceptibility of the negro is, however, much less than that of the white race, and among those attacked the mortality, as a rule, is small. This is shown by the statistics relating to white and black troops in the British service at West India stations. "While in Jamaica the annual loss among the former amounts to 102 per 1,000 of the mean strength, the deaths among the black did not exceed 8 per 1,000. In the Bahamas the mortality of the whites was 59 in 1,000, that of the blacks, 5.6 in 1,000" (La Roche).

In the report of the board of experts appointed by Congress to investigate the epidemics of 1878, we find the following remarks: "Berwick City, 40 cases among colored, no deaths. Morgan City, 21 cases among colored persons. Brownsville, Tenn., of 162 colored cases 21 died. Chattanooga, of 685 cases, 256 whites, 429 colored; of 164 deaths, 118 whites, 46 colored. Decatur, Ala., of 64 white cases 28 died, of 168 colored 21 died."

The indigeneous races of the West Indies and of the continents of North and South America have no immunity, except such as is acquired by residence in an endemic focus of the disease, and the same is true of the Mongolian race; but like the negro they have, although to a less degree, less susceptibility than the white race, and the mortality among those attacked is not so great.

In general, it may be stated that the natives of northern latitudes are more susceptible than those born in tropical or subtropical climates. Blair, who had an extended experience in Guiana, says: "The lower the winter temperature in the native country of those attacked the more severe was their sickness, so that while the mortality among West Indians amounted to only 6.9 per cent. of the sick, it rose to 17.1 among the Italians and French, 19.3 among the English, 20.2 among the Germans and Dutch, and 27.7 among Scandinavians and Russians."

Barton gives the following figures, showing the mortality per thousand among

different races, and those of the same race from different latitudes, in the city of New Orleans in the great epidemic of 1853:

	Per 1,000.
Native creoles	3.58
Strangers from—	
West Indies, Mexico, and South America	6.14
Southern States of the Union	13.22
Spain and Italy	22.06
Middle States of the Union	30.69
New York and New England States	32.83
Western States of the Union	44.23
France	48.13
British America	50.24
Great Britain	52.19
Germany	132.01
Scandinavia	163.26
Austria and Switzerland	220.08
Netherlands	328.94

SEX.

There is probably no difference in the susceptibility of the sexes, but males are attacked in greater proportion than females, because they more frequently and often recklessly visit infected localities. The mortality is, as a rule, considerably greater among males. Ligon, in giving an account of the pestilence at Barbadoes in 1647, of which he was an eye-witness, says: "The cause was unknown; one could not say if the ships of commerce had imported the scourge, or if it came from bad food, marshy water, the intemperance of the colonists, and, above all, the great quantity of eau-de-vie which they drank. * * * It was the most debauched who perished first, and not one woman died for ten men." No doubt Ligon was right in ascribing the difference in the mortality of the sexes largely to the difference in their habits, with reference to the use of eau-de-vie. Those who habitually use spirituous liquors are less likely to recover from an attack than the temperate, and a recent debauch is a recognized predisposing cause. Sailors who go on shore at an infected port for "a little spree" very commonly turn up in the hospital, or are taken sick after they come on board ship, and serve as the starting point of an epidemic among their comrades, and subsequently perhaps at the port of destination of the vessel. The greater prevalence and severity of the disease in epidemics among males has been noticed by numerous authors, and has been verified in the writer's personal experience.

AGE.

Infants and old persons enjoy a comparative immunity, due in part, no doubt, to the fact that they are less exposed than active individuals in middle life. Dr. Rush records the fact that he has "met with a violent case of the disease in a child of four months, and a moderate case in a child of ten weeks" (La Roche). Very young infants, however, commonly escape, or suffer so mild an attack that the nature of the disease is not recognized. In cities like New Orleans, which have suffered repeated epidemics, the proportion of children attacked is often exceptionally large, because they constitute a large share of the unacclimated population, having been born since the last epidemic. Dr. Bemiss has given the following table, showing the number attacked and the comparative mortality for different ages, in the great epidemic of 1878.

The results of private practice in New Orleans are exhibited in the following statistics. Four of the principal practitioners in the city treated, in private practice, 975 patients—909 white, and 66 colored. Of the former, 92, or 10.11 per cent., died;

of the colored only 2 died. The cases and deaths among the whites, classified by age, are as follows:

Age.	Cases.	Deaths.	Per cent.
Under 5 years of age	206	26	12.67
From 5 to 10 years of age	233	20	8.61
From 10 to 20 years of age	183	9	4.9
From 20 to 40 years of age	232	39	16.7
From 40 to 60 years of age	47	6	12.7
From 60 to 80 years of age	4	2	50.0

This table does not support the statement that adults are more likely to be attacked than children, but it must be remembered that it relates to cases occurring in private practice, in a community in which the adults were largely protected by previous attacks, or by passing through repeated epidemics.

In a review of the mortality in the same epidemic, with reference to age, Dr. C. B. White arrives at the following conclusions:

"First. That the mortality of boys at 4 years is not because a very much larger number of boys were taken sick, but that there is an actual greater mortality.

"Second. It is seen that, though the deaths decline with great rapidity—being at 4 years 344; at 5 years, 169; at 6 years, 65, the cases do not decrease in the same ratio, but decline at follows: Cases at 4 years 822; cases at 5 years, 740; cases at 6 years, 624; the recoveries being proportionally much larger.

"Third. From 7 to 11 years of age the death rate remains uniform, the disease being comparatively much less fatal. According to Dowler, the mortality among children in the epidemic of 1841, in New Orleans, was very small. On the other hand, in the epidemic of 1853, in the same city, it was considerable."

Dr. Charles Delery has given the following table, compiled from the official reports in the office of the board of health, showing the mortality during the epidemic of 1867, among children born in the city of New Orleans:

Age.	Males.	Females.	Total.
Below 1 year	9	9	18
1 to 2 years	22	18	40
2 to 3 years	18	23	41
3 to 4 years	19	10	29
4 to 5 years	13	9	22
5 to 6 years	15	10	25
6 to 7 years	24	5	29
7 to 8 years	10	9	19
8 to 9 years	9	1	10
Total	139	94	233

Total deaths from July 29 to November 7, 1867: Males, 167; females, 133 (of these 5 colored, 2 males and 3 females).

Dr. Henry Smith, Marine Hospital service, reports that in the epidemic at Shreveport, La., in 1873, out of a total of 584 deaths in which the age was ascertained "100 died under 10 years of age, 93 died between 10 and 20 years of age, 156 died between 20 and 30 years of age, 134 died between 30 and 40 years of age, 59 died between 40 and 50 years of age, 29 died between 50 and 60 years of age, 13 died above 60 years of age."

IMMUNITY.

Immunity is acquired by suffering an attack of the disease, or by long residence in localities where it is endemic or prevails frequently as an epidemic; this acquired immunity is not, however, absolute.

Second attacks no doubt occasionally occur, although this has been denied by some authors. Blair, whose experience was very great, says that he does not believe there is an instance of a second attack after a month's perfect restoration to health. Other authors are equally positive in their statements. On the other hand, we have numerous authentic accounts of second attacks. Thus "Dr. Jackson states that in Spain, during the epidemic of 1820, 20 well-authenticated instances came within his knowledge of persons being attacked who had had the disease before." Dr. Wragg, speaking of the epidemic in Charleston in 1854, reports the occurrence of second attacks in a number of instances, and says: "Six of these were so well proved as to admit of no doubt on the subject. Some of the patients were identified as having gone through the fever in this (the Roper) hospital in 1852, throwing up black vomit on both occasions." (La Roche.) Dr. Rush has given evidence of the same kind, and says that a second attack was more common when the first had been comparatively mild.

Dr. Delery, in his account of the epidemic of 1867 in the city of New Orleans, gives 3 cases of second attacks, 1 fatal, in which the first attack occurred in a previous epidemic in the same city, and was vouched for by experienced physicians known to him. While, then, it can not be denied that second attacks occasionally occur, the evidence of experienced observers in all parts of the yellow fever zone is opposed to the view that this is a common occurrence. Those who have considered yellow fever nothing more than a grave form of malarial fever, an idea which was entertained by numerous physicians in this country and in the West Indies in the early part of the present century, very naturally failed to differentiate the disease from the endemic malarial fevers which they encountered, and believed that it might recur an indefinite number of times.

ACCLIMATIZATION.

It is a remarkable fact that the population of a large city like Havana, or Rio Janeiro, in which yellow fever has been endemic for a series of years, enjoys such a degree of immunity from the effects of the deadly poison that there is no interruption of business or pleasure at a time when strangers in the city are falling sick on every side. The development of an *epidemic* in these cities depends upon the presence of susceptible strangers in sufficient number to furnish a series of cases considered large enough to justify the use of the word. The presence of but few strangers during the epidemic season leads to the announcement that the disease is not epidemic, but that sporadic cases occur from time to time. Under exceptional circumstances, however, epidemics are developed in these endemic foci of the disease, in which those who, by birth or long residence, were supposed to be acclimatized furnish a certain quota to the general mortality. This has frequently occurred, for example, in the city of New Orleans, where yellow fever formerly prevailed almost annually, and where the creole population was supposed to enjoy an hereditary immunity. Dowler, who has made a special study of the question, says of the creole population of New Orleans:

"A few physicians and others, mostly advocates of the contagiousness of yellow fever, maintain that all the creoles of New Orleans, not less than strangers, have this disease once during life, for the most part during childhood, and that it proves fatal to many of them."

"This sweeping statement, however, is with few exceptions erroneous, as may be proved by authentic documents concerning all of the epidemics witnessed by the writer for 17 years, not excepting the extraordinary one of 1853 itself."

"It will have been remarked by careful observers that many families have settled in New Orleans for half a lifetime without ever having had yellow fever. Indeed, it

has been thought by many physicians, previous to 1853, that at least one-third of all strangers settling permanently in New Orleans escaped yellow fever altogether."

"The exemption of the creolized of the city is a fact which every epidemic has confirmed; for example, take that of 1841, in which 1,800 died, 5 of whom only were natives of the city, 1 aged 3 weeks; 3, 2 years. In 1843, among 692 deaths from yellow fever but 2 are certified as having been born in New Orleans."

The writer quoted believes, with many other physicians residing in endemic foci of the disease, that immunity is, "to a great degree, hereditary, or transmissible from parents to children." This is generally accepted among the physicians and the native population of the city of Havana; but there is reason to believe that it is a mistake, and that the creole child owes his immunity not to his parents, but to individual acclimatization, and not infrequently, to say the least, to a mild, unrecognized attack of yellow fever. Dr. Dowler says that "many creole children had, during the epidemic of 1853, a fever, a slight fever, yellow fever if you please, known as such rather by the coexistence of the epidemic than from any severe symptoms among these children, a slight fever never yet described, having generally but one paroxysm, lasting from six hours to one, two, or three days, scarcely ever requiring medication. That a few of these cases acquired an alarming violence, and even proved fatal, is most true, most deplorable."

Hinemann writes with reference to Vera Cruz: "Until lately the physicians and people of Vera Cruz supported with fanaticism the dogma that natives were absolutely exempt from yellow fever. But the fearful epidemics of recent years (1875, 1877, 1878) have worked a change; for so many native children and adults suffered that the truth could no longer be denied that these do not enjoy an absolute immunity."

In Cuba the dogma that creoles are exempt from yellow fever did not withstand the searching investigation made by the Havana yellow-fever commission of 1879. We quote some of the evidence collected and reported by Dr. Chaille, president of this commission: "Dr. Navea, of San José de las Lajas, an inland town some 20 miles southeast of Havana, presented the following interesting report after it had received the full approval of Drs. Cabrera and Bofil, his colleagues at San José: 'We have here annually, in the practice of the three physicians, from twenty to thirty Cuban children and from thirty to forty Cuban adults attacked with bilious remittent fever, which is popularly designated typhus. There is nothing whatever to constitute a differential diagnosis between this fever of the natives and the yellow fever of strangers. It is characterized by its hemorrhagic tendency, albuminuria, black vomit, and all the symptoms of yellow fever. It is so well marked that even when seen by the uneducated they exclaim, "Vomito!" The treatment for the one is best for the other. We have never seen a second attack of this bilious remittent fever, nor one who had recovered from it attacked with yellow fever. If any one of us three physicians here sees this fever attack a native Cuban we say "bilious remittent fever," and if it attacks a person not a native of Cuba we say "yellow fever;" but at bottom it is the same disease, and we agree to call it bilious remittent fever in Cubans solely because these believe themselves exempt from yellow fever, and are so prejudiced that they would be alarmed if assured their disease was really yellow fever."

Dr. Mantiguazi, of Cienfuegos, reported as follows: "During December, 1875, the sanitary condition improved, but a certain fever has prevailed among children, which is known here as typhus, although it resembles in nothing the disease to which Europeans give this name, and which so often occurs in camps. By this fever I have lost one patient, a child 8 years of age, born in this town. It presented all the symptoms of yellow fever, for on the second day this patient had the characteristic vomit and stools, and died on the third day. In a consultation with three other physicians they agreed with me in my diagnosis, with this difference, that they said that these same symptoms which constituted yellow fever in strangers constituted in natives typhus. I have been told that eight to ten children have died of this disease."

Dr. Mazarredo, a graduate of Paris, who had had 20 years' experience in Cuba, wrote as follows: "In my own practice I have seen cases of yellow fever in children from 1 to 5 years of age, and even not over a year of age, in whom it has been fatal, and I am now well convinced that children born in Cienfuegos are exactly in the same conditions the first years of their lives as are other newcomers and just as liable to its attacks. Nevertheless I consider that children are generally less prone to suffer severely. * * * Worthy practitioners of this locality give the name of typhus fever to these cases, and although they admit that no difference whatever exists between the symptoms, march, and duration of this compared with yellow fever, still they think the former a swamp fever, and hence more amenable to quinine."

Blair, who has written a classical account of the epidemic of 1851–'54 in British Guiana, says that "infancy was one of the most favoring causes of the action of the yellow-fever poison. The constitution of the new-born or young white creole was highly susceptible. He or she was truly in the category of newcomers."

Recent experience at Key West (epidemic of 1887) shows that the children of acclimated Cubans, born since the arrival of their parents at Key West, have the same susceptibility to the disease as other children born of native (creole) parents. We can not, therefore, admit that inherited immunity has been established.

On the other hand, we are not prepared to assert that there is no immunity independent of an attack of the disease. Unrecognized mild attacks in adults, and especially in the negro race and among creole children, are no doubt of frequent occurrence. But it can not be denied that independently of any febrile manifestations individuals of all ages who have resided in an infected locality for some time acquire a comparative immunity, which increases with length of residence and degree of exposure to the action of the specific cause of the disease.

It is generally conceded that this acclimatization is lost, or at least reduced to a considerable extent, by residence for some years in a latitude outside of the "yellow fever zone." And some authors maintain that the immunity due to an attack of the disease is in like manner lost by a protracted absence from its accustomed haunts. The writer's observations are opposed to the latter statement. In the epidemic at Fort Barrancas, Fla., in 1875, in which nearly every unprotected person who remained in the infected area suffered an attack, three officers, who had previously had yellow fever, remained in perfect health, although they had all resided for several years in a northern locality since the date of their attack—one for more than 20 years.

Hinemann, who has had an extended experience at Vera Cruz, says that "even foreigners may remain insusceptible to the disease for a considerable number of years, provided they do not leave the focus of the disease during that period. An absence of a few months only is sufficient to take away this immunity.

The fact that foreigners may remain for years in Havana, in Rio Janeiro, or other endemic foci of the disease without suffering an attack is undeniable. I found this especially to be the case in Rio, where there is a large foreign population. It is true that some who have escaped for a series of years often fall sick at last in a season of unusual epidemic prevalence, but comparative immunity is shown by the fact that, as among the creole population, the disease is not so fatal with them as among newly arrived strangers. The effects of acclimatization in large cities, like Rio Janeiro and Havana, are illustrated by the fact that in these cities yellow fever is, for the native population, a disease of minor importance. This is shown by the following tables.

In Rio, a city having a population of 400,000, the mortality from some of the principal causes of death is given in the official report of the superior board of health, as follows, for the year 1886, which was considered an epidemic year so far as yellow fever is concerned:

	Mortality.
1. Tuberculosis	2,077
2. Diseases of the circulatory apparatus	1,458
3. Diseases of the cerebro-spinal apparatus	1,345
4. Diseases of the digestive apparatus	1,097
5. Malarial diseases	1,086
6. Yellow fever	1,015

In Havana, the principal causes of death among the civil population, in the year 1880, are given officially as follows:

	Whites.	Colored.
1. Phthisis pulmonalis	941	503
2. Diarrhea and enteritis	450	186
3. Yellow fever	426	.....

That acclimatization is not due to the influence of climate, but is an acquired tolerance to the action of the yellow-fever poison, is shown by the history of this disease in Rio Janeiro. At the time of its introduction, in 1849, it found an unprotected population, and for a series of years prevailed as an epidemic among this population, causing a mortality quite comparable to that in similarly located cities in other parts of the world when first invaded by the scourge. At present the native population furnishes but a comparatively small proportion of the total number of deaths by this disease.

PREDISPOSING CAUSES.

Plethora is considered by many physicians, in the regions where yellow fever prevails, to be a predisposing cause, and to account for the greater susceptibility of strangers from northern countries. *Constipation* is generally regarded as conducive to an attack. Other predisposing causes are, *fatigue* from excessive exertion, *debility* resulting from a recent debauch, or from any other cause, *exposure* to the direct rays of the sun, violent mental emotions, such as *grief* or *fear*, and, in general, any influence capable of depressing the vital powers, or any disturbance of the normal functional activities of the body.

MODE OF INFECTION.

Yellow fever is contracted by exposure in infected localities, and not directly by contact with the sick. This is established by a mass of evidence which is recorded in the literature of the subject, but is still denied by some physicians, who regard it as a contagious disease in the same sense that smallpox and measles are contagious. In this respect it is like cholera and typhoid fever. There is something given off from the body of the sick by which, when external conditions are favorable, new centers of infection are established; and it seems probable that, as in the diseases mentioned, this germinal principle of the disease is contained in the alvine discharges of the sick. That it is not given off from the general surface of the body is an inference which we base upon the established fact that the disease is not transmitted directly from individual to individual. It is true that the contagionists bring forward a class of facts which, regarded alone, seem to give some support to their views, but we believe these facts to be explicable in accordance with the statements above made. Persons who successively fall sick in the same house, or on board ship, are not infected one from another, but contract the disease from a common source, the infected premises, or ship. Indirectly, of course, they contract the disease through the agency of the individual, or fomites, through whom the house or ship first became infected.

Formerly the battle between the contagionists and the noncontagionists was one involving the question of local origin on one side, together with a strenuous denial of the transmissibility of the disease and the value of quarantine restrictions. The contagionists, on the other hand, insisted upon the exotic origin of the disease, and its transmissibility by ships and persons. And to this extent they were right. There can be no doubt that the prevalence of the disease in the United States depends upon the introduction of an exotic germ; but it also depends upon local conditions which favor the development of this germ. The yellow fever patient, however many germs he may carry in his intestines or elsewhere, does not directly endanger those who

come near him any more than a gelatine stick—culture of the spirillum of Asia cholera, or of the anthrax bacillus places in danger the student of bacteriology who is engaged in studying it.

It is well known to the people of the city of Mexico that a visit to the seacoast city of Vera Cruz during the epidemic season is likely to result in an attack of yellow fever. It is also well established that those who fall sick with the disease after their return to the city of Mexico never communicate it to others who are closely associated with them as attendants, etc. The same is true at the health resort, Petropolis, located in the mountains, within a few hours' ride of the city of Rio Janeiro. Frequently individuals fall sick at Petropolis who have visited the infected city of Rio. Never do they communicate the disease to others. This is also the experience of the physicians in charge of hospitals—*e. g.*, the Charity Hospital of New Orleans. So long as the hospital and its vicinity remain uninfected, cases do not originate in the hospital, although yellow fever patients may be admitted to the wards with unacclimatized persons suffering with other diseases, and be cared for by susceptible attendants.

In his report upon the camps established near Memphis in the epidemics of 1878 and 1879, Colonel Cameron makes the following statement: "It was found necessary that the officer in authority should set an example of constant indifference to attack in order to appease, as far as possible, the constant anxiety of the population under his charge. Especially was this true in 1878, as depopulation went on slowly that year, and infected people poured daily into the camps from the more pestilential portions of the city. Very many reached camp with the fever on them, so that as many as seventeen persons fell victims in one night, not a few in their tents. *In no instance, however, did they communicate the disease to their families or bedfellows* as far as could be traced."

In the same epidemic (1878) Dr. Minor reports that over thirty cases were discovered among refugees in Cincinnati, Ohio, and says: "No physician or nurse contracted the disease, and in no instance did it exhibit any tendency to spread." The same was true in Nashville the same year. Twenty imported cases occurred in different parts of the city without any local cases resulting from them (Report of Nashville Board of Health). Evidence of this kind could be extended to fill a volume, but sufficient has been presented to establish the statement made, and the reader may be referred to the "Proofs of Noncontagion," in the second volume of the classical work of La Roche (pp. 236–566).

We have already, in discussing the nature of the specific cause of the disease, referred to the fact that there is no satisfactory evidence that the disease is contracted by the use of contaminated water, as is the case in cholera and typhoid fever. We quote the following conclusions of the board of experts appointed to investigate the epidemic of 1878.

(6) "Yellow fever is a disease of singular local attachments. It often becomes epidemic in one section of a city, and sometimes a very small section of it, while it fails to present itself at all in other sections of the same city, and in these localizations it exhibits a remarkable indifference to topographical and social surroundings."

(7) "In the dissemination of yellow fever, atmospheric air is the usual medium through which the infection is received into the human system."

ETIOLOGY OF EPIDEMICS.

The development of an epidemic of yellow fever in places removed from the endemic foci of the disease depends upon: (*a*) the introduction of the specific cause by yellow fever patients, or through infected articles—fomites; (*b*) local conditions which favor the multiplication of the specific germ external to the body; (*c*) favorable meteorological conditions; (*d*) the presence of susceptible individuals in the infected locality.

We quite agree with the board of experts above quoted in the following conclusions also: (31) "The most frequent agency in the dissemination of yellow fever

from place to place is found in yellow fever patients; and more epidemics of yellow fever have resulted from the introduction into previously exempt places of persons sick of the disease, or falling sick after arrival, than from all other causes. To what extent the body of the sick person is responsible for this result, and to what extent his clothing and baggage is responsible for it, is not known."

The last clause in the above quoted conclusion shows that the board of experts admitted the possibility that the yellow fever patient acts simply as a carrier of the infectious agent about his person or in his baggage. The fact that he has yellow fever is evidence that he comes from an infected locality, and he may be instrumental in establishing a new center of infection for this reason, rather than because he is himself a victim to the disease. This is a possibility which should not be lost sight of; but, reasoning from analogy and from known facts it seems extremely probable—indeed we may almost say certain—that the sick establish new centers of infection because the infectious agent is reproduced in their bodies and is contained in their excreta.

That infected centers may be established independently of the arrival of sick persons is, however, beyond question. A striking instance of this is afforded by an outbreak which occurred in Madrid in 1878. A circumscribed epidemic was developed in this city about the 1st of September, which resulted in a mortality of 35 out of 50 cases taken sick. This outbreak was traced to importation, although all of the cases occurred among the permanent residents of the infected area. Associated with the young people who first fell sick, crowded in the same rooms with them to the number of 10 or 15 in a room, were a number of soldiers recently returned from Cuba, *with their baggage*. These men had themselves suffered from yellow fever in Cuba, or were acclimatized by long residence there.

We have on record instances which appear to be authentic, of the development of an epidemic as a result of the opening of a trunk containing *infected clothing*, sent from a locality where the disease was prevailing to one previously healthy. Epidemics have also been traced to the unloading of *earth ballast* from the shores of an infected port upon the wharves of a healthy place in the yellow fever zone.

The first cases of local origin in an epidemic do not, as a rule, occur until some time has elapsed after the arrival of the infected ship or fomites or sick person responsible for the introduction of the "germ." This interval may vary from a few days to several weeks, according as local conditions are favorable or otherwise for the development of the infectious agent.

In the great epidemic of 1878, which was traced by Dr. Choppin, president of the Louisiana State board of health, to importation by the steamship *Emily B. Souder*, which arrived from Havana on the 23d of May, the first cases of local origin did not occur until after an interval of 5 or 6 weeks. But these first cases occurred, according to Dr. Choppin, in the immediate vicinity of the houses in which two of the officers of the *Souder* (Clarke, the purser, and Elliott, one of the engineers) died soon after the arrival of that vessel.

The *local conditions* which favor the development of the exotic germ are various: (*a*) *Latitude*. Our account of the geographical limits of the prevalence of the disease suffices to show the influence of latitude, which appears to be simply a question of temperature. (*b*) *Altitude*. The facts do not justify the conclusions that the limitations as to altitude depend solely upon the lower temperature of elevated regions. As pointed out by Hirsch, "the disease stops short at many points in the West Indies, where the climate is still in the highest degree tropical. On the other hand, there have been epidemics in cool weather at very considerable altitudes, as, for example, at Newcastle, in Jamaica" (elevation about 4,000 feet). In the Antilles the disease has rarely appeared at a height of more than 700 feet. In Mexico it has prevailed at Cordova (2,500 feet), but it is unknown in the cities of Orizaba, Jalapa, and Puebla, which have an elevation of more than 3,000 feet. In Spain a single limited outbreak has occurred at Madrid, which is about 2,000 feet above the sea level; but with this exception the altitudinal range has rarely exceeded 1,000 feet. In the United States

the most elevated locality in which the disease has prevailed as an epidemic is Chattanooga, Tenn., which is 745 feet above the sealevel. (*c*) Yellow fever is essentially a disease of the seacoast, and while in great epidemics it may become widely diffused in the interior, it follows, for the most part, the course of *navigable rivers*. (*d*) It is a disease of cities and towns of considerable size, and rarely extends to small country villages, or among the scattered rural population. (*e*) In seaport towns it frequently makes its first appearance in the vicinity of the wharves, or in localities frequented by sailors. (*f*) Above all it is a disease which is influenced by *local unsanitary conditions*. In those places where it is endemic it haunts the low-lying and filthy portions of the town, and in epidemics it exhibits a marked preference for towns which are in an unsanitary condition. It frequently happens that when a town is invaded the disease is limited, for a considerable time at least, to the filthy portions of the place, in which the degraded victims of poverty and vice congregate in ill-ventilated apartments, surrounded by the filth which accumulates in such localities when not kept under a rigid sanitary supervision. (*g*) *Decomposing organic matter of animal origin* seems to furnish an especially favorable nidus for the germ. This is shown by its favorite haunts and by the fact that in marshy places in the vicinity of cities where it prevails, and where vegetable decomposition is active, it does not effect a lodgment. On the other hand, the influence of putrefying organic matter of animal origin in the production of epidemics has several times been made apparent.

Dr. Parkes, the famous English hygienist, maintained the fecal origin of the disease, and there seems to be good reason for the belief that the accumulation of this kind of filth in exposed situations is favorable to the development of an epidemic.

Conditions relating to *soil and geological formation* have not been shown to influence in an essential manner the development or diffusion of the disease. On the other hand, the numerous epidemics which have occurred on shipboard show that the disease is quite independent of such conditions, and at the same time disprove the theory, so vigorously maintained by numerous authors during the first half of the present century, that the disease is due to emanations of the same nature as those which produce the so-called "malarial fevers." *Meteorological conditions* control in a most decided manner the prevalence of the disease in localities where it is endemic, and its epidemic extension when new infected centers are established among a susceptible population. The influence of *temperature* is shown by the fact that it is a disease of the tropics and of hot seasons; that it prevails throughout the year in the cities of Rio Janeiro, Havana, and Vera Cruz, although to a much less extent during the cool season; while in more temperate regions its prevalence is limited to the summer season. It does not prevail as an endemic disease in places which have a mean winter temperature much below 65°, and as a rule epidemics are not developed at a lower temperature than 75° to 80° F. The approach of cool weather checks the progress of an epidemic, and it is arrested completely when the temperature falls to the freezing point. There are, however, numerous facts which indicate that the infectious agent is not destroyed by a freezing temperature, although rendered inactive. Epidemics which have been checked by frost have been revived by the recurrence of warm weather, and in certain instances in temperate regions the germ has survived the winter, and a second epidemic has occurred without a new importation (Memphis, 1878–'79; Cadiz, 1800–'01; Malaga, 1808–'09). Epidemics which originate early in the season often terminate before there is frost, simply because the susceptible material is exhausted; but when strangers venture within the infected area they furnish evidence of the continued activity of the morbific poison by falling victims to the disease. The influence of *season* is shown by the following tables:

Mortality from yellow fever in Rio Janeiro during the year 1886.

Month.	Total deaths.	Mean temperature.	Total rainfall.
		°	
January	135	77.5	1.03
February	234	76.3	10.94
March	347	77.5	3.02
April	220	75.0	8.81
May	48	69.2	0.35
June	18	67.3	1.68
July	9	65.5	1.55
August	2	65.5	5.37
September	0	68.5	4.59
October	1	69.2	1.37
November	0	72.9	1.37
December	1	74.1	8.92
Total	1,015	.....	49.00

Mortality from yellow fever in Rio Janeiro from January, 1851 *to July,* 1870. (*Hirsch.*)

Epidemic season.		Nonepidemic season.	
January	1,118	July	242
February	1,760	August	164
March	1,732	September	108
April	1,434	October	104
May	996	November	116
June	557	December	223
Total	7,597	Total	957
89 per cent.		11 per cent.	

Mortality from yellow fever in the city of Havana for ten years, 1870 *to* 1879, *inclusive.* (*Chaille, report to national board of health.*)

Month.	1870.	1871.	1872.	1873.	1874.	1875.	1876.	1877.	1878.	1879.	Average of ten years.
January	6	18	20	32	7	16	24	8	26	11	17
February	4	23	13	23	4	16	24	9	13	13	14
March	4	12	4	27	18	32	29	11	5	6	15
April	6	54	4	37	22	34	33	8	28	13	24
May	14	91	13	127	85	32	103	16	53	40	57
June	66	201	68	378	172	142	292	143	184	237	188
July	112	234	68	416	361	187	675	249	504	475	328
August	201	138	70	127	416	144	250	285	374	417	242
September	91	72	59	35	186	102	97	234	179	148	120
October	77	55	38	28	91	109	42	185	106	44	78
November	49	51	85	5	42	105	31	150	53	31	60
December	35	42	73	9	21	82	19	76	34	9	40
Total	665	991	515	1,244	1,425	1,001	1,619	1,374	1,559	1,444	

The above tables show us that temperature is only one of several factors which control the prevalence of the disease, for with a tolerably uniform temperature during a series of years, at Vera Cruz, for example, we have some years marked by a

very considerable mortality, *e. g.*, 1875, 1881, and others, in which but few cases occur, even during the hottest part of the season, *e. g.*, 1869, 1870, 1879. Again, it happens that after a summer of almost complete exemption an epidemic is developed in the autumn which runs through the entire winter (1880–'81). An important factor which does not usually appear in statistical tables relates to the number of susceptible individuals present at different times. The arrival, for example, of emigrants or of bodies of unacclimated troops at Havana or Vera Cruz would almost inevitably be followed by a marked increase in the mortality from yellow fever.

Although the development of an epidemic seems to require a comparatively high temperature (75° to 80°), experience shows that it may continue in full force at a much lower temperature (60° to 70°) when local conditions are favorable and a susceptible population is exposed in the affected area. A good example of this is given by the epidemic at Chattanooga, Tenn., which city was invaded for the first time in 1878. We obtain our data from a paper by Dr. J. H. Vandeman, late of the Marine Hospital Service.

The first imported case, a refugee, was taken sick August 17, died August 21. The first case among the residents of the city occurred over 3 weeks later (September 13), and proved fatal on September 19. The further progress of the epidemic and the mean temperature for each week are shown in the following table:

Epidemic at Chattanooga, Tenn., in 1878.

Week ending—	New cases.	Deaths.	Mean temperature.
September 27	40	21	79.71°
October 4	47	19	70.42
October 11	144	22	66.66
October 18	99	33	63.14
October 25	74	21	56.52
November 1	30	12	48.66
November 8	9	4	53.04
November 15	2	2	51.83

Here we have an epidemic, which was at its acme when the temperature was below the mean temperature of the winter months in Rio Janeiro, Havana, or Vera Cruz. The monthly mean temperature in the last-named city, and the mortality for each month for a period of four years, are given in the following table:

Mortality in the city of Vera Cruz for four years.

Months.	1878.		1879.		1880.		1881.	
	Deat h	Mean temperature.	Deaths.	Mean temperature.	Deaths.	Mean temperature.	Deaths.	Mean temperature.
January	16	69.5°	6	72.9°	2	73.2°	28	67.3°
February	5	72.0	4	72.8	0	75.3	22	67.4
March	0	76.0	2	78.9	1	78.1	29	75.4
April	1	80.5	1	79.0	1	80.8	29	77.1
May	7	84.3	1	83.4	0	84.9	94	82.5
June	58	86.9	1	83.5	0	86.4	233	84.6
July	114	85.6	2	85.6	1	87.6	132	88.1
August	110	84.6	1	86.5	3	86.2	39	87.6
September	62	83.4	3	80.7	9	83.3	22	84.3
October	45	81.2	0	79.0	42	77.5	25	80.6
November	24	77.3	0	79.0	92	76.7	18	75.3
December	7	70.2	0	73.4	98	74.7	4	73.6

An inspection of the above table shows that after an epidemic in the summer of 1878, which may be said to have lasted from June to December, two seasons of comparative immunity followed, which can not be ascribed to a lower temperature. Indeed, the average temperature for the six months from April to September, inclusive, was somewhat less in 1880 than in the epidemic years 1878 and 1881. Again, we see that in 1880, after an unusually healthy summer, yellow fever prevailed to a considerable extent during the months of October, November, and December, although the temperature was less than during the corresponding months of the preceding year. The epidemic impetus continued during the following year, attaining its maximun of intensity in the months of May, June, and July, although in two of these months (May and June) the temperature was lower than in the corresponding months of the preceding healthy summer.

Another factor of importance in the etiology of yellow fever epidemics is atmospheric *moisture* and *precipitation*. Yellow fever is a disease of the seacoast and of the margins of great rivers, and it does not prevail in arid, desert places in the interior, although the elevation may be but little above the sea level, and the temperature extremely high. There is reason to think that this difference is largely due to the difference as to the moisture in the atmosphere and in the soil. Some authors have insisted especially upon the presence of moisture in the atmosphere almost to the point of saturation as an essential condition for the development of an epidemic. But, on the other hand, epidemics have occurred in unusually dry seasons, as at New Orleans in 1841. In Martinique, according to Dutrouleau, yellow fever has sometimes committed its greatest ravages after and during the dryest seasons.

La Roche states that the epidemics in Philadelphia were "connected in most instances at least with a deficiency of atmospheric and terrestrial humidity—though in all instances of its occurrence it will be found that, prior to the accession of dry weather, the earth had been more or less saturated with rain." As pointed out by this author, "the coexistence of a clear atmosphere is often seen." The fact seems to be that, while a certain amount of moisture is essential, the limits vary considerably. In general, it may perhaps safely be said that the degree of humidity and the temperature most favorable for the rapid decomposition of organic material, which forms the nidus in which the infectious agent multiplies, are the most favorable for the epidemic extension of the disease.

Heavy rains, by purifying the air and cleansing the streets and sewers of an infected city, exercise a favorable effect upon its sanitary condition, and in the tropics the commencement of the rainy season often puts an end to the prevailing epidemic. It is probable that the statement made by some authors, that upon the coast of Guiana, and elsewhere in the tropics, dry weather is favorable to the spread of the disease, is but another way of stating the fact that the heavy rains of tropical regions are unfavorable, the dry weather being only dry by comparison.

In the north temperate zone southerly winds are favorable to the progress of an epidemic, because of the elevated temperature which accompanies them, and, on the contrary, northerly winds have a tendency to arrest it. Fresh sea breezes, and especially the trade winds in the tropics, by reason of their constancy, are beneficial from a sanitary point of view. They dilute and carry away the poisonous emanations from the foul and narrow streets of infected cities and refresh and invigorate the population.

There is no evidence that the infectious agent of yellow fever can be conveyed by the wind in an active condition to any considerable distance, and the wind has but little to do with the dissemination of the disease. In cities the extension of an epidemic from centers, resulting from the importation of cases or fomites, is usually quite gradual and independent of the prevailing currents of air. The board of experts appointed by Congress to investigate the epidemic of 1878 arrived at the following conclusion:

"We know of no instance, either from our own observations or from the published

records of yellow fever, in which it has been established that the disease has been carried to any considerable distance by atmospheric currents, or by any modes or vehicles of conveyance other than those connected with human traffic and travel."

In the comparatively small and landlocked harbor of Havana vessels which anchor some distance from shore and which are kept in a good sanitary condition do not suffer from yellow fever unless unacclimatized members of the crew are permitted to go on shore.

Vessels lying at the wharves, on the contrary, are very likely to become infected.

There are, however, numerous instances in which vessels have become infected from the shore or from other vessels, or in which yellow fever has appeared in places lying to the leeward of infected vessels, in which the transmission of the disease has been ascribed to the agency of the wind. It is difficult to decide to what extent this explanation is correct, for we would have to be sure that no direct communication had occurred before accepting it; and the possibility that the infection may be conveyed to the shore by infected articles thrown overboard, in the case of infected vessels anchored to the windward of healthy places, must be borne in mind.

PROPHYLAXIS.

What has been said as to the etiology of yellow fever indicates clearly enough the measures of prophylaxis to be taken in localities subject to invasion. These are: (*a*) *Exclusion* of the exotic germ of the disease by the sanitary supervision, at the port of departure, of ships sailing from infected ports, and their thorough disinfection at the port of arrival when there is evidence or a reasonable suspicion that they are infected; (*b*) *isolation of the sick* on shipboard, at quarantine stations, and, so far as practicable, in recently infected places; (*c*) *disinfection* of excreta, and of the clothing and bedding used by the sick, and of localities into which cases have been introduced or which have become infected in any way; (*d*) *depopulation* of infected places, *i. e.*, the removal of all susceptible persons whose presence is not absolutely necessary for the care of the sick.

The *time quarantine* of former days, which proposed to exclude the disease by detaining ship and passengers at a quarantine station for a fixed period of time after its arrival, irrespective of its sanitary condition, and if yellow fever occurred on board to quarantine the vessel for a certain number of days after the occurrence of the last case, has been found unreliable and has been pretty generally abandoned. The modern method of exclusion which relies upon the sanitary supervision of the ship at the port of departure and, so far as practicable, while in transit, and upon isolation of the sick and disinfection of the vessel when cases of yellow fever have occurred on board at a quarantine station provided with the appliances for doing this in an effective manner, has proved far more successful. Reliance upon a time quarantine is unsafe for the reason that the vessel may remain infected for an indefinite period of time after the occurrence of cases on board, or even independently of any case, if she has been at the wharves of an infected port. No more cases occur after all of the passengers and crew susceptible to the disease have suffered an attack, and it may happen that there are no susceptible persons on board; but the vessel is none the less infected and dangerous to the inhabitants of the port which admits her to pratique without a thorough disinfection. On the other hand if we are to trust to the measures indicated the sooner they are put into execution the better.

Depopulation of infected ships or towns, when yellow fever makes its appearance during the epidemic season, is a measure of great importance, which should be carried out, when practicable, under proper medical supervision. It has often happened, especially upon naval vessels, that the well are retained upon the infected ship and the sick sent to a hospital on shore, under the idea that the chief danger lies in contact with the sick. This is a serious mistake, and has resulted in the loss

of many lives. In the United States Army it is well understood that all susceptible well persons are to be removed to a healthy locality as soon as yellow fever is known to have effected a lodgment in quarters occupied by troops. It has been found by experience that a move into camp checks the progress of the disease among the garrison, although this may be established but a few miles from the infected barracks.

In the case of towns in a country subject to invasion by the disease depopulation often occurs in a way which is most dangerous to other communities. It very often happens that the nature of the disease is not at first recognized even by the physicians, and when recognized the hope is cherished that it will not become epidemic until a considerable mortality, or the death of some prominent citizen, arouses the fears of the people and a general "stampede" occurs. The attempt has often been made to shut in the people of an infected town by means of a "sanitary cordon," composed of armed guards. This procedure is not only inhumane, so far as the susceptible inhabitants of the place are concerned, whom it is proposed to compel to remain within the infected area, but it is, as a rule, futile; for when a frightened man desires to escape from an unseen enemy it is difficult to keep him in a place not surrounded by insurmountable walls, or by an impassable barrier of some kind.

At the International Sanitary Conference of Rome (1885) the following resolution was adopted, with a single dissenting vote—that of the delegate from Turkey:

6. "Land quarantines and sanitary cordons are useless."

This related, it is true, to cholera, but it applies as well in the case of yellow fever. Another resolution, adopted at the same conference, is to this effect:

84. "The measures recommended against cholera are, in general, applicable to yellow fever and to other diseases which prevail in epidemic form, under the influence of bad sanitary conditions, and which are transmitted by human intercourse.

"The most effective measures for preventing the propagation of diseases of this class are:

"The sanitary improvement of cities and of vessels sailing from infected ports, isolation of the sick, and disinfection of infected or suspected articles and localities."

In yellow fever we require, in addition to this, depopulation of the infected localities whenever it is practicable, and this should be effected systematically, and with due precautions to prevent the transportation of infectious material to other places.

Of all measures of prophylaxis, those which relate to the sanitary improvement of cities and towns liable to become infected are perhaps the most important. Municipal hygiene has made great strides since the early part of the present century, and it is probably to this fact, more than to any other, that certain Northern cities which formerly suffered severely from yellow fever epidemics owe their long immunity from such visitations—*e. g.*, New York and Philadelphia.

Individual prophylaxis requires the individual, first of all, to avoid infected localities. If it is absolutely necessary for a susceptible person to visit a place where yellow fever is prevailing, or to remain in one in which it has effected a lodgment, he should observe the following precautions: Keep away from low-lying and filthy portions of the city; avoid the vicinity of the wharves, and all localities known to be centers of infection, especially at night; sleep as far from the ground as possible; avoid excesses of all kinds, and especially in the use of alcoholic drinks; keep out of the sun during the hottest part of the day, and be careful not to become overheated by violent exercise; avoid constipation.

With reference to the method of prophylaxis by inoculation, practiced in Brazil by Dr. Domingos Freire, and in Mexico by Dr. Carmona y Valle, the writer, after a careful examination, has reported officially that—

"There is no satisfactory evidence that the method of inoculation practiced by Dr. Domingos Freire has any prophylactic value.

"The claims of Dr. Carmona y Valle, of Mexico, to have discovered the specific cause of yellow fever have likewise no scientific basis, and he has failed to demonstrate the protective value of his proposed method of prophylaxis."

INCUBATION.

The period of incubation in yellow fever does not usually exceed 4 or 5 days, and may be less than 24 hours. Instances of a much longer period of incubation have been given by various authors—even as long as 6 weeks or 2 months—but we are satisfied that these are due either to error in diagnosis or to the fact that the cases resulted from the establishment of a new and unrecognized center of infection. Those who believe that the disease is only communicated by personal contagion naturally date the exposure to the latest date when such personal contact was possible. Thus, when a first case occurs on a ship at sea, two or three weeks after leaving an infected port, the period of incubation is supposed to be at least this long. On the contrary, the attack is due, in all probability, to the fact that the ship is infected, and the first case will probably be quickly followed by others which have no direct connection with it, but result, as it did, from exposure on the infected vessel. Instances of an attack occuring within 24 hours after arrival in a city where the disease was prevailing as an epidemic are numerous and well authenticated. Dr. Rush says in his account of the epidemic of 1793: "The seeds of the disease, when received into the body, were generally excited into action in a few days. I met with several cases in which they acted so as to produce a fever on the same day on which they were received into the system." During the epidemic at Gibraltar, in 1804, strangers were in several instances attacked on the second or third day after landing, and some are said to have been seized on the first day. According to Drs. Pariset, Balby, and François, the period of incubation at Barcelona, in 1821, appeared very short. "We have," they say, "very strong reasons to suspect, and these reasons are founded on facts, that this period does not exceed 24 hours, or 3 days at most." (La Roche.)

In the epidemic at Fort Barrancas, Fla., in 1875, the writer had an opportunity to fix the limits of the period of incubation. The whole garrison was exposed in the infected locality, and a number of cases had occurred when the command was removed into camp in a healthy place across the bay, near Fort Pickens. On the day following the removal 7 cases were sent back from the camp for treatment in hospital. The next day 11 cases came from the camp, the following day 2, and the fifth day 2. The remnant, consisting of 48 individuals, remained in good health until a month later, when 2 cases again occurred in camp. We do not believe that these 2 cases represent a prolonged period of incubation, but suppose that in the interval the camp had also become infected.

It is proper to state that several authors who have had great experience believe that the period of incubation may be extended to 14 or even more days. (Blair, Rush, Feraud.)

CLINICAL HISTORY.

As a rule, an attack of yellow fever is not preceded by any well-marked *premonitory symptoms*. The attack may occur at night in one who went to bed in his usual state of health, or in the early morning, after an uninterrupted sleep, or during the day, while engaged in ordinary occupations. In other cases there is a feeling of lassitude and discomfort for two or three days prior to the attack, with loss of appetite, slight pain in the back and loins, a feeling of giddiness or slight headache, flatulent eructations, constipation, a tendency to perspire at night or upon very slight exertion, and more or less muscular debility, together with a disinclination for any mental exertion.

Dr. Wragg, who had charge of the Roper Hospital, at Charleston, during the epidemic of 1854, made particular inquiries with reference to premonitory symptoms, and states that "out of a total of 225 cases the attack was sudden in 92; in 32 it came on insidiously, the patient complaining of malaise, etc., for a considerable time; and that 101 offered the usual symptoms characterizing the approach of fever.

The attack is commonly inaugurated with a more or less decided chill, which by its violence and duration affords some indication of the probable severity of the case. In certain grave forms of the disease, however, the onset is insidious, and is not marked by any perceptible chill. In a considerable proportion of the mild cases also, especially in the tropics, there is no rigor, and the patient experiences at most only a slight sensation of coldness, which quickly gives place to that of heat. If a thermometer is placed in the axilla during the initial chill it will be found that the temperature is already considerably above the normal, and very frequently it reaches the highest point obtained during the entire attack within a few hours from its inception.

Accompanying the chill are other nervous phenomena, similar in kind to those attending the onset of other specific febrile diseases. There is *cephalalgia*, often very severe, and located by preference in the forehead and supraorbital region; the eyeballs also are painful, and there is intolerance of light in some instances. *Pain in the loins* is a very constant and early symptom, which occasions much distress, and sometimes extorts groans and cries from the patient. At the same time pain is usually experienced in the lower extremities, often of a very severe character, constituting the *coup de barre* of the French authors; it affects especially the calves of the legs, the knees, and the ankles. These symptoms continue and are even aggravated after the rigor has passed and the febrile stage is fully developed. In the meantime the *face* becomes flushed and sometimes deep red and swollen in appearance; the *eyes* are shining and suffused, the conjunctivæ more or less hyperæmic and often deeply injected, in severe cases presenting a fiery, inflamed appearance, which is accompanied by photophobia; the *skin* becomes hot and dry and there is apt to be, especially in patients of a nervous temperament, great *restlessness* and *jactitation*.

Systematic authors have described numerous varieties of the disease, but these are for the most part simply different grades in the degree of severity, or depend upon individual peculiarities and complications. Yellow fever is the same in all parts of the world where it occurs, and every great epidemic furnishes examples of the several varieties which have been described. It will suffice here to mention the classification adopted by one or two standard authors. La Roche gives an account of the clinical history of the disease under the following headings: Inflammatory species, including three grades, intense, mild, and ephemeral; congestive species, including four grades, aggravated, adynamic, walking, and apoplectic. Beranger-Feraud, in his elaborate account of the disease as it prevails at Martinique, classifies the cases as follows:

First degree.—Mild yellow fever.

Second degree.—Yellow fever of moderate intensity: (*a*) cases in which the onset is frank; (*b*) cases in which it is insidious.

Third degree.—Grave yellow fever: (*a*) ordinary forms, including the gastric, adynamic, ataxic, congestive, and typhoid forms; (*b*) rare forms, including the hyperesthetic, gangrenous, algid or choleraic, and hydrophobic.

Fourth degree.—Yellow fever *sidérante*.

Different epidemics are sometimes characterized by the predominance of one or the other of the forms described, and the character of the disease as to severity often varies greatly during the same epidemic. The earlier cases are sometimes mild and the mortality small, while later the greater intensity or malignancy of the poison is shown by the occurrence of a large number of cases of the severest grade, and even of those rapidly fatal attacks denominated by the French *sidérante*.

It is evident, from the account given of the symptoms manifested at the outset of an attack, that these are not sufficiently characteristic to determine the nature of the disease, and in the absence of a prevailing epidemic its early recognition will depend largely upon other facts relating to the antecedents of the patient, etc. The complete clinical history of a case, however, gives a tableau which is easily recognized by one who is familiar with the disease. In this clinical history the thermometric observations form a very important item.

Yellow fever is a disease of a single febrile paroxysm, lasting from 48 hours to 7 or 8 days, more commonly from 3 to 5 days. The acme of temperature is reached at the outset, and from this time the temperature line is a descending one, interrupted sometimes by a slight evening exacerbation, up to the termination of the first period of the disease—*febrile stage*. The second stage is characterized by great prostration of the vital powers and lasts from a few hours to 2 or 3 days—*stage of calm*. The temperature during this stage sometimes remains a degree or more above the normal, but more commonly it is normal or even subnormal for a time. This is followed in severe cases by a *reactionary fever* of irregular duration which presents a more or less remittent character. These features are shown in the accompanying temperature charts.

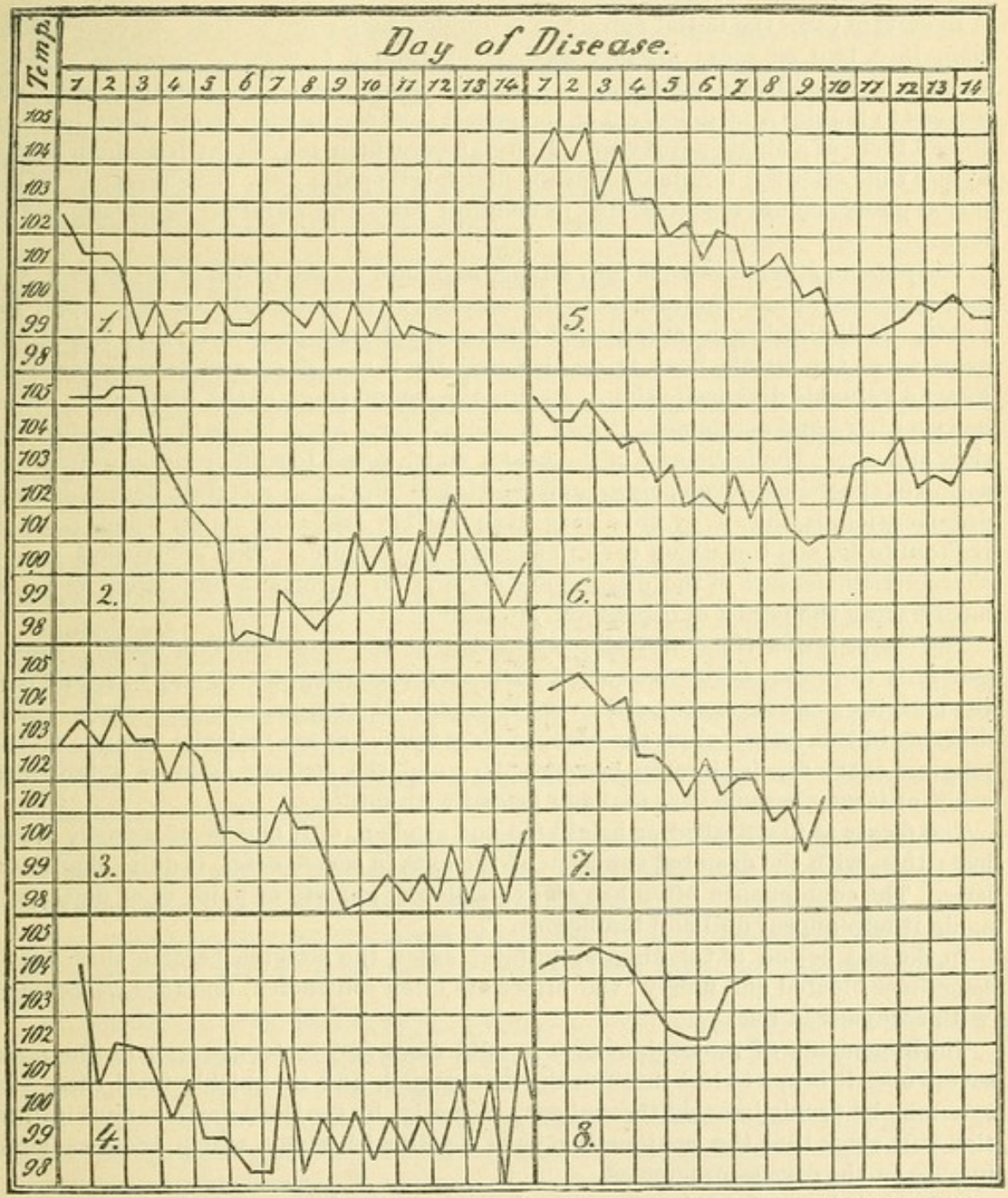


FIG. 4668.—Temperature Curve of Yellow Fever. 1. Mild case of brief duration: male, aged 30; Fort Barrancas, Fla., 1873 (Sternberg). 2. Typical severe case, recovered; male, aged 27; Fort Barrancas, Fla., 1873 (Sternberg). 3. Protracted mild case, recovered; male, aged 27; Fort Barrancas, Fla., 1873 (Sternberg). 4. Typical case of moderate severity, recovered; male, aged 31. Fort Barrancas, Fla., 1873 (Sternberg). 5. Protracted case of moderate severity ("*degré moyen*"), recovered (Bérenger-Féraud). 6. Typical severe case; protracted; death on 13th day (Bérenger-Féraud). 7. Fatal case: death on 9th day; male, aged 28; Fort Barrancas, Fla., 1873 (Sternberg). 8. Fatal case, "*marche rapide*" (Bérenger-Féraud).

Naturally the typical temperature curve is disturbed by complications—visceral congestions, abscesses, parotitis, etc. It is also disturbed by indiscretions in diet, overactive medication, and by moral causes (especially fright and grief). In mild

cases the acme of temperature is reached during the first 2 or 3 hours of the attack. In more protracted and severe cases it is not reached until the second or third day, rarely later. In an analysis of 192 cases recorded by Faget, Jones, and myself, the acme was reached on the first day in 102, on the second in 54, on the third in 33, and on the fourth in 3. The temperature rarely, if ever, exceeds 108°. The highest temperature recorded by Faget was 107.2°. Thornton, in a total of 143 cases occurring at Memphis, noted a temperature of 108 in a single instance. With this exception, 106.5° is the highest temperature recorded by him. In my own observations 106° has been the highest temperature noted. The temperature often rises rapidly just before death, and a very high post-mortem temperature (108° to 110°) is a common phenomenon.

In certain cases the initial paroxysm is divided by a more or less complete remission, into two or more distinct periods of from 2 to 4 days' duration. These cases, which possibly depend upon a malarial complication, are not sufficiently numerous to require a modification of the general statement that yellow fever is a continued fever of a single paroxysm. In relapses, which may occur from imprudence at any time after the termination of the first febrile paroxysm, the characters of the initial paroxysm are repeated, but in nonfatal cases the duration is usually not so long.

The *pulse* in sthenic cases is full, strong, and hard at the outset of the attack, and may reach 120 or more pulsations in the minute, more commonly not more than 100 to 110. It diminishes in rapidity and force as the disease progresses, and this occurs even when the febrile heat is not reduced for 2 or 3 days, and is considered by Faget a valuable diagnostic sign. During the second stage of the disease the pulse, however hard and accelerated it may previously have been, becomes preternaturally slow and soft. The feebleness of the heart, which seems to suffer especially from the action of the yellow-fever poison, and which has undergone a certain degree of fatty degeneration, is shown by this very compressible and slow pulse, which is often reduced to 40, and sometimes even to 30 beats per minute. This constitutes a very characteristic feature of the disease, and affords an important indication for treatment during the period of depression, or "calm."

The *tongue* is sometimes but slightly coated at the outset, and is usually moist; generally it quickly becomes covered with a white coating, which may be in streaks; the margins as a rule remain red; very commonly the tongue is narrow and pointed, differing in this respect from the broad, flabby tongue of the malarial fevers. In the progress of the disease it often becomes dry, and the coating assumes a brownish color, or it may become very foul and loaded with sordes.

The *face* is at first flushed or bright red and swollen, or it may be of a dusky violet hue; this, with the deep red suffusion of the eyes in severe cases, is quite characteristic. The countenance often has an expression of anxiety or pain, or of dejection; again it may appear dull and indifferent.

In the last period of the disease, in fatal cases, the features become shrunken—sometimes bloated and flabby; the brows are often contracted, and the eyes sunken, with ecchymosed lids.

The hyperæmia of the conjunctivæ in mild cases may be temporary; in the more severe ones it is apt to last through the first period, and in quickly fatal cases the eyes may be deeply injected throughout. Usually, by the third day a careful inspection will show that the conjunctivæ have a yellowish tinge, which becomes more intense as the disease progresses.

The *skin* is hot and dry in some cases throughout the first period; in others it soon becomes moist and there is a tendency to free perspiration, which is readily induced by covering with blankets, warm drinks, etc. Even in those cases in which the skin is hot and dry the termination of the first period is marked by a soft, cool, and usually moist surface. In exceptional cases the skin remains hot and dry up to the fatal termination. When death occurs in the stage of depression, the surface becomes cold

and often is covered with a clammy sweat. Many authors have spoken of a peculiar odor given off from the surface of yellow-fever patients, and various attempts have been made to define its character by comparison with other known odors. Dr. Rush said that it resembled that of the "washings of a gun." Dr. Jackson describes it as "sickly and faint, and not unlike the smell of a fish market."

The color of the skin, which has given name to the disease, is not always seen, but usually a yellow discoloration begins to make its appearance toward the end of the first period, and later becomes more intense, lasting for some time after convalescence is established. It varies much in intensity, from a slight yellow tinge to a deep orange or saffron color. In certain cases the skin presents a mahogany color, or that of bronze. In fatal cases the yellow color is developed immediately after death, even if it has not been very noticeable before dissolution took place.

The proportion of cases in which this yellow color of the skin is observed varies greatly in different epidemics, and even in different periods of the same epidemic. Dr. Rochoux states that in the West Indies it is absent in about half of the cases that recover. Berenger-Feraud, referring to this statement, says that there are in fact two kinds of icterus in yellow fever, the one due to blood pigment, denominated by Professor Gubler *hæmapheique;* the other to bile pigments, and called by the same author *bilipheique.* The first is considered by Feraud to be constant and characteristic; it occurs at a time when the urine is albuminous and free from bile, and manifests itself about the beginning of the second stage by a yellow tinge of the conjunctivæ, of the face, and of the skin over the great vessels. It coincides with the period during which the hæmorrhagic tendency of the disease manifests itself. The other form of icterus appears at the end of the second period, or during convalescence, at a time when the urine contains a notable quantity of bile pigments; it gives rise to the deep orange or saffron color of the skin which is so striking, but which only occurs in a certain proportion of the cases, and can not be considered an essential character of the disease. We are disposed to think that Feraud is quite right in this account of the icterus of yellow fever. A slight yellow discoloration of the conjunctivæ can commonly be detected during the second stage, even in mild cases in which no discoloration of the skin is perceptible.

Various skin eruptions have been described as occurring occasionally, but there is nothing characteristic about any one of them unless it is the erythematous eruption about the scrotum which Berenger-Feraud believes to be pathognomonic of the disease. Other eruptions mentioned as occurring occasionally are petechiæ, vesicular and pustular eruptions, livid spots and vibices, erythematous patches about the knees and elbows, or a general erythematous eruption, papular eruptions, pustules about the mouth, furuncles, etc.

The *urine* in yellow fever, even during the first period, is reduced in amount below the normal standard. This marked reduction, and in fatal cases very commonly complete suppression, is a notable feature of the disease.

The presence of albumen in greater or less amount is a symptom which is so constant that it has come to be generally accepted as one of the pathognomonic features of the disease.

The diminution in the amount of the urinary secretion in connection with the amount of albumen present is of importance in a prognostic sense, as it is to a certain extent an index of the gravity of the attack.

The most marked diminution occurs during the stage of depression, and the few ounces secreted during the 24 hours in severe cases are loaded with albumen to such an extent as to form coagula which occupy one-half to two-thirds of the contents of the test tube.

In a series of observations made at Fort Barrancas, Fla., in 1875, to ascertain the amount and specific gravity of the urine in nonfatal cases of yellow fever, the writer arrived at the following results:

The amount considered in connection with the *specific gravity* is, of course, an

index of the total solids excreted. In the table we have eliminated from the specific gravity column the constant factor, 1,000. The amount for the first day is no doubt too small, on account of the urine having been only partly collected. It will be noted that the amount increases, while the specific gravity diminishes, from the outset of the attack up to the time of complete convalescence.

Taking the average of sixteen non-fatal cases, I obtained the following results:

[Amount in fluid ounces × specific gravity — 1,000.]

	Amt. S.G.
First day	8 × 23 = 184
Second day	11.5 × 25 = 287
Third day	16 × 28 = 448
Fourth day	18 × 22 = 396
Fifth day	19 × 22 = 418
Sixth day	20 × 22 = 440
Seventh day	22 × 21 = 462
Eighth day	22 × 19 = 418
Ninth day	23 × 16 = 368
Tenth day	28 × 13 = 364
Eleventh day	37 × 11 = 407
Twelfth day	41 × 13 = 533

It will be seen that the product of the amount, multiplied by the specific gravity, is tolerably uniform from the third to the twelfth day, and that this uniformity is preserved by a daily falling off of the specific gravity to compensate for the daily increase in quantity. To ascertain the product of amount multiplied by specific gravity in patients convalescent from yellow fever at a later date, I had the urine passed by sixteen convalescents, on full diet, who had been out of bed from ten to twenty days, preserved and measured. The result was that the average of amount, multiplied by the specific gravity for the sixteen cases, was 491—a product but little in excess of that obtained during the continuance of the fever when the patients were quiet in bed. The figures in the table may then be taken as representing, approximately, the normal quantity of urinary solids which should be excreted daily during the progress of an attack of yellow fever, and if the amount falls materially below these figures, defective excretion may be premised, and treatment and prognosis governed accordingly.

In mild cases, only a slight trace of *albumen* may be found in the urine for a day or two, but usually the deposit is sufficiently abundant after the second day to leave no doubt as to its character; it becomes more abundant at the termination of the first stage, and in severe cases throughout the stage of calm, at which time suppression is very liable to occur, especially if, as a result of exposure, the cutaneous transpiration is checked. The early appearance and abundant presence of albumen is generally recognized as an evil prognostic. The reappearance of albumen, after it has once disappeared, indicates a relapse.

The amount of *urea* eliminated by the kidneys is less than normal, and in inverse proportion to the severity of the attack. According to Cunisset, the quantity is generally in proportion to the amount of urine secreted, and when the amount increases it is a favorable symptom. Uric acid is also said by this author to be present in diminished quantity, but in much less proportion than the urea. He says: "We have seen urine containing only 7 grammes of urea per litre give a relatively abundant deposit of uric acid." Bile pigments usually appear in the last days of sickness, and their presence is generally considered a favorable prognostic sign. In exceptional cases the urine may contain blood, from renal or vesical hemorrhage. The urine in yellow fever almost invariably presents a decided acid reaction.

The symptoms connected with the *nervous system* are varied. In mild cases the intellect remains undisturbed, and only a moderate amount of transient pain in the

head and back marks the onset of the attack. In severe cases the frontal headache and rachialgia are most distressing, and may last throughout the first period of the disease. This stage is also one of great restlessness and jactitation; the patient sleeps but little, and his sleep is apt to be disturbed by distressing dreams; the mind often seems to be in a state of tension, the patient is watchful, excited, and anxious. In other cases the mind is calm, and in others, again, there is a condition of apparent apathy or indifference. *Delirium* is not a very common symptom in yellow fever, and the intellect often remains unclouded throughout, even in fatal cases. Some cases, however, are attended with incoherency of ideas, or hallucinations, and in some there is active delirium. More frequently the mind falls into a torpid condition, the patient is somnolent, and when awakened is disposed to be taciturn. A certain number of fatal cases are characterized by active delirium, followed by coma, and a greater number by coma gradually developed. In a limited number of cases death is preceded by convulsions, and occasionally tetanic symptoms of more or less general character have been noted. Among the nervous symptoms may be mentioned deep sighing respiration, sometimes of a spasmodic character. Naturally the *respiration* is increased in frequency during the febrile stage; in the stage of calm it again becomes normal in mild cases, or sighing and spasmodic in those of a graver character.

There is complete *anorexia* during the first stage of the disease, but when the remission occurs patients are very likely to desire food, and even to insist upon having it. Thirst is a constant symptom during the febrile stage, and also in the second period, especially when there is frequent and copious vomiting—black vomit. The bowels are commonly somewhat constipated at the outset. In the second period of the disease there is apt to be more or less diarrhea, and in fatal cases a dark fluid is often discharged from the bowels, resembling precisely that from the stomach. Occasionally there is a discharge of pure blood, the result of intestinal hemorrhage. A choleraic form of the disease is sometimes met with in which there is profuse diarrhea and collapse, similar to that in the algid stage of Asiatic cholera.

Symptoms connected with the *stomach* form a prominent part of the clinical history of yellow fever. The characteristic black vomit which has so much occupied the attention of medical authors is for the vulgar the most striking feature of the disease, and even many physicians reserve their diagnosis in early cases during an epidemic until they have had ocular evidence that the vomited matter is black. The common name of the disease in Spanish countries—vomito—refers to this symptom. Yet in a majority of the cases there is no vomiting of black matter. Indeed, this is recognized as a very grave symptom, and although a considerable number of recoveries occur after the characteristic black vomit has been ejected, this may be considered an exception to the general rule.

Vomiting is a common symptom during the first period of the disease; sometimes the fluid ejected has a yellow color from the presence of bile, but more frequently it is colorless and consists only of the fluids ingested, containing, in suspension, a little mucus from the stomach; it is almost always acid. In favorable cases vomiting ceases with the first period of the disease; in those of a graver character, after an interval, perhaps, of 24 hours or more, during which there is more or less gastric distress, or a feeling of weight and discomfort, vomiting again occurs, either of a clear acid fluid or of one of the varieties of black vomit. At first the black material may be in the form of little flocculi suspended in a transparent fluid, "coffee-ground vomit," and as the case progresses the amount of this material increases, until the whole fluid appears to be uniformly black. Upon allowing it to stand, however, it commonly separates into two portions, and it will be seen that it still consists of a clear liquid with the black matter in suspension. Upon microscopic examination it will be found that the colored material is made up of little masses which are not black, but have a yellowish-brown color. A careful examination of recent specimens shows that these little masses contain decolorized and more or less deformed red blood cor-

puscles and granular leucocytes, and that the brown matter is diffused about these agglomerated cellular elements and the matrix of mucus in which they often seem to be included. There is no doubt that the black vomit is due to passive hemorrhage from the gastric mucous membrane, although this has been disputed by some recent authors—Freire, Carmona, Gibier. Formerly it was maintained by some that the black matter was a secretion from the stomach, and by others that it was "vitiated black bile." I have myself repeatedly verified the fact by microscopic examination that it contains the cellular elements of the blood. In certain cases there is vomiting of pure blood, resulting from more active gastric hemorrhage. This black vomit is not always discharged during life, but it is extremely rare not to find it in the stomach after death.

The fact that the gastric mucous membrane is seriously involved in this disease is shown not alone by the vomiting, the passive hemorrhage, and the post-mortem appearances, but also by the constant feeling of discomfort or pain in severe cases, and by a marked tenderness upon pressure over the epigastrium.

Hemorrhage may occur not only from the mucous membrane of the stomach and intestine, but also from that of the mouth, nose, bladder, and uterus, and even from the eyes or ears. In some epidemics epistaxis is quite common; it may occur during the first period of the disease, but is more frequent during the second, at which time the general tendency to hemorrhage is developed. Next to epistaxis, hemorrhage from the buccal mucous membrane, and especially from the tongue, the gums, and the lips, is the most frequent.

In mild cases of yellow fever, convalescence is quickly established. Instead of the usual depression which marks the second stage of the disease, the patient, after a fever of 3 to 5 days' duration, may enter almost immediately into convalescence, and within a week may be ready to resume his usual avocations. But in severe cases, convalescence is often slow and may be interrupted by various *complications*—parotitis, buboes, furuncles, abscesses, nepatitis, diarrhea. In the army, experience shows that but few of those attacked are able to resume their duties in less than 10 days, and a considerable number remain in hospital from 30 to 50 days. Blair found the average number of days during which patients were retained in hospital after convalescence was established to be 6.55 for mild cases, and 7.91 for grave cases.

Relapses occur for the most part during the early period of convalescence, or before it is fairly established; they are generally regarded as even more dangerous than the first attack. Occasionally a relapse, or second attack, occurs as late as from 2 to 4 weeks after the termination of the febrile period of the first attack.

PROGNOSIS.

The prognosis in yellow fever should always be guarded, for not only is it a very fatal disease in its severer forms, but cases which appear mild at the outset may suddenly assume a grave character. It is more favorable in the case of women and children than with men, and is especially unfavorable in those of intemperate habits, and in plethoric persons who have recently arrived in the infected locality. The principal guide in forming a prognosis at the outset of a case is furnished by the temperature observations. When the body heat does not go above 103° to 103½° F. during the first two days, a favorable result may be anticipated. Later, the amount and character of the urinary secretion is the most important prognostic indication. When this is scanty and heavily loaded with albumen, the case is very grave, although the general appearance of the patient may be favorable, and he may insist that he is "all right" and wants something to eat. On the other hand, hopes of a favorable issue may be entertained even in cases characterized by great prostration, and in which the hemorrhagic tendency is pronounced, if the urinary secretion is tolerably abundant and the quantity of albumen small. The throwing up of black vomit is by no means a fatal sign, but as a rule the passive hemorrhages which occur

during the second stage of the disease are of grave import. Epistaxis occurring during the first stage has been considered by some authors a favorable event (?).

The early appearance of jaundice is an unfavorable sign; as is also frequent vomiting and great distress and tenderness in the epigastric region. Intense and long continued injection of the conjunctivæ, giving the eyes a fiery-red color, is unfavorable. Delirium, great restlessness and jactitation, and sighing respiration, are all symptoms which give reason for anxiety as to the result.

The prognostic indications as furnished by temperature observations are shown in the following table, made by the writer, some years since, from a series of cases in which a complete and careful record had been made:

Cases in which the temperature was—	No. of cases.	No. of deaths.	Percentage of death to cases.
107° and above	13	13	100
106°—107°	9	9	100
105°—106°	36	22	61
104°—105°	80	24	30
103°—104°	87	6	*7
102°—103°	29	0	....
101°—102°	15	0	....
Total	269	74	27.5

*Nearly.

MORTALITY.

The mortality from yellow fever varies greatly in different epidemics and among different classes of the community.

Among the natives of cities in which the disease is endemic, or in which it has frequently prevailed as an epidemic, it may be as low as from 7 to 10 per cent. One reason for this comparatively small mortality is found in the fact that a considerable proportion of the cases in such a community are among children. Among unacclimated adults the mortality ranges from 20 to 60 per cent., and under certain circumstances even exceeds the latter figure. In the great Philadelphia epidemics of the last and the beginning of the present century, the mortality was from 20 to 80 per cent. In the French Antilles, according to Dutrouleau, the mortality during the years 1851 to 1857, inclusive, ranged from 12.9 per cent. to 50 per cent. Blair gives the mortality at the Seaman's Hospital at Georgetown (Demerara) in 1838 as 19.5 per cent.

At Vera Cruz the mortality for 7 years (1875–1881) according to Dr. Molina's statistics, was 41.78 per cent. in the hospital for men, and 41 per cent. in the hospital for women. In Rio Janeiro the mortality for the year 1870 was 17.4 per cent. In the epidemic of 1873 it was 23.3 per cent.

The mortality among the Spanish troops in Cuba, according to the statistics of Dr. Bastarreche, was, in the year 1855, 24.31 per cent., and in the royal navy 17.8 per cent.

Dr. Bemiss gives the mortality rate for the city of New Orleans, during the great epidemic of 1878, as 16.66, and classifying the cases according to age, has constructed a table which we have used on a preceding page (p. 52).

The day of death is given by Berenger-Feraud as follows, in a total of 1,059 cases occuring in the French Antilles:

Died on the second day, 16—1.5 per cent.; third day, 56—5.3 per cent.; fourth day, 141—13.4 per cent; fifth day, 165—15.7 per cent.; sixth day, 177—16.9 per cent.; seventh day, 151—14.3 per cent.; eighth day, 89—8.5 per cent.; ninth day, 42—3.9

per cent.; tenth day, 35—3.3 per cent.; eleventh day, 28—2.6 per cent.; twelfth day, 34—3.2 per cent.; thirteenth day, 25—2.3 per cent.; fourteenth day, 21—1.9 per cent.; fifteenth day, 8—0.7 per cent.; sixteenth day, 7—0.6 per cent.; seventeenth day, 13—1.2 per cent.; eighteenth day, 6; nineteenth day, 6; twentieth day, 7; twenty-first day, 3; twenty-second day 2; twenty-third day, 5; twenty-fourth day, 3; twenty-fifth day, 3; twenty-sixth day, 5; twenty-seventh day, 1; twenty-eighth day, 1; twenty-ninth day, 2; thirtieth day, 1; thirty-first day, 3; thirty-second day, 1; thirty-third day, 1; thirty-sixth day, 1. This table shows that 67 per cent. of the whole number died during the first week, and 82 per cent. during the first 10 days. In a similar table constructed by Dr. B. A. Clements, of the United States Army, out of a total of 111 fatal cases, death occurred, during the first week in 73 per cent.; during the first 10 days in 82 per cent.

DIAGNOSIS.

Notwithstanding the well-marked features of a typical case of yellow fever, it very often happens that the early cases in an epidemic are not recognized, and even in fatal cases, with the assistance of an autopsy, physicians not previously familiar with the disease often differ as to the diagnosis. At the outset of an attack, indeed, there is nothing sufficiently characteristic in the symptoms to enable even an expert to decide definitely that the case is yellow fever, although there are certain indications which may give him a strong suspicion that it is. The flushed face, fiery red eyes, pointed tongue, supraorbital pain, rachialgia, etc. When such a case occurs during the epidemic prevalence of yellow fever, or in a stranger at one of its endemic foci, the inference usually is that the individual has yellow fever; but this inference is sometimes a mistake, and more than one stranger is sent to the yellow fever hospital (Jurajuba), of Rio Janeiro, who turns out to have some other febrile complaint, sometimes one of the eruptive fevers. A case in one of our own seaport cities, with precisely the same symptoms, occurring at a time when yellow fever was not prevailing, would almost infallibly be pronounced "malarial fever," or "bilious fever," or "remittent fever," as soon as it was evident that it was not one of the specific eruptive fevers. And this diagnosis would very probably be maintained if no evidence could be found that the individual had been exposed to yellow fever, even if the further clinical history of the case corresponded with that of this disease. It is in this way that a series of cases, occurring perhaps in the practice of several different physicians, at the outset of an epidemic are frequently called by some other name. The fact that the early cases in an epidemic are sometimes of a mild character adds to the liability to error. A man has a chill followed by fever, and receives in the next 48 hours several full doses of quinine; at the end of this time his fever has disappeared, and with the exception of a certain amount of debility, and perhaps an irritable stomach, he feels quite well. The inference is that he had an attack of malarial fever which was cut short by the treatment; perhaps it was so, but the inference is the same in either case. I have seen cases in the hospitals of Vera Cruz, for example, in which there was a slight amount of albumen in the urine, and in which the depression and slow pulse following a single febrile paroxysm of brief duration seemed to me to be characteristic of a mild attack of yellow fever, but in which the diagnosis was malarial fever, and the brief duration of the attack was ascribed to the full doses of quinine which had been administered. I have seen precisely similar cases during the prevalence of an epidemic of yellow fever, in which no quinine was given, and in which, notwithstanding, the febrile paroxysm was of brief duration and did not return. For example, the case represented by tracing No. 1 in the chart. This case by itself could not have been diagnosed as yellow fever, but occurring as it did with several others of the same mild character, side by side with severe and fatal cases of this disease, I did not doubt that it was due to the same specific cause, and was in fact a case of yellow fever.

The occurrence, in a southern seaport, of a group of cases of ephemeral fever, in a particular locality, especially among the colored population, should always arouse suspicion and lead to investigation. When in such a city, or in any place within the limits of yellow-fever invasion, and during the summer months, we hear that a "malignant form of malarial fever" has appeared in a certain limited area, a single house, or square, or district, we shall not be apt to go wrong in the inference that the disease is yellow fever. Malignant forms of malarial fever belong to the country rather than to the city, and cases do not occur in groups within city limits, where the malarial poison is not generated in its most intense form, if at all. Formerly, very much uncertainty as to diagnosis arose from mistaken ideas as to the etiology of the disease. A considerable number of physicians in the United States maintained that yellow fever and the malarial fevers are closely allied if not identical diseases, due to the same general causes, and the idea was very prevalent that, under the influence of meteorological conditions, a mild form of malarial fever might be transformed into the malignant yellow fever. Under this idea, physicians felt justified in calling the mild cases in an epidemic malarial fever, and in reserving the name yellow fever for those in which a yellow skin, highly albuminous urine, and black vomit make up the clinical tableau to which they gave the name yellow fever. Of these three prominent features, only one is a constant character which can serve in establishing the diagnosis in mild cases. This is the presence of *albumen* in the urine. At some period of the disease, even in the mildest cases, there will be a distinct trace of albumen in the urine, as shown by the usual tests, and this will generally be sufficiently abundant to leave no doubt in the mind of the observer as to the nature of the precipitate. In 61 cases occurring during an epidemic in Jamaica, Donnet found albumen present for the first time: In 2 cases on the first day; in 11 cases on the second day; in 19 cases on the third day; in 14 cases on the fourth day; in 6 cases on the fifth day; in 4 cases on the sixth day; in 4 cases on the seventh day; in 1 case on the eighth day. It will be seen that in by far the larger number of cases the presence of albumen was verified on the second, third, or fourth day. The value of this test in the differential diagnosis of yellow fever is indisputable. It is true that a trace of albumen is sometimes found in the urine of severe cases of fevers of malarial origin, but in cases of yellow fever of equal severity, as compared with these, the precipitate would, as a rule, be very abundant on the third or fourth day of sickness, forming a deposit to the extent of one-fourth to one-half the contents of the test tube, or even more. At the same time, a microscopical examination would show the presence of numerous granular casts from the tubuli uriniferi.

These are found also, although in a smaller number, in the urine of the milder cases during the second stage of the disease. For diagnostic purposes the character of the febrile paroxysm, and especially the phenomena following this, which mark the second stage of the disease, are the most important. The characteristics of the temperature curve are sufficiently shown by the charts of typical cases in page 67, It must be remembered, however, that while the stage of depression is commonly marked by a normal, or even subnormal, temperature, in a considerable number of severe and fatal cases the temperature still remains a degree or two above the normal when the cool skin and slow, soft pulse would indicate, if the thermometer were not used, that there was complete apyrexia; or again, the skin remains dry, and the patient falls into a typhoid condition, with a fluctuating temperature, without having had a complete remission of febrile heat. The second stage of the disease is, however, commonly well marked in nonfatal cases, and is its most characteristic feature; the remarkably slow and soft pulse, the evident prostration of the vital powers, although the patient may be comfortable and even cheerful, and desirous of getting up, or of taking food; the yellow tinge of the conjunctivæ and skin—not always present; the tenderness on pressure in the epigastric region, and often a feeling of weight and distress in this region, attended with intense thirst and vomiting of a transparent acid fluid, or of the characteristic black vomit; the

tendency to passive hemorrhage from the mucous surfaces of the mouth or nose; oozing of dark blood from the gums, or lips, or sides of the red and fissured tongue; the scanty urinary secretion and the presence of albumen, usually in considerable amount; all of these symptoms constitute an *ensemble* that for the practiced eye and hand is unmistakable.

The differential diagnosis from bilious remittent fever, with which yellow fever has frequently been confounded, is not always possible at the outset of an attack, but the contrast in the complete clinical history of the two diseases is sufficiently striking. Remittent fever, as the name indicates, is characterized by periodical remissions of the febrile heat; this is, however, in severe cases often not well marked so far as exact thermometric observations are concerned, and is rather a remission, usually occurring in the morning, of the general symptoms—headache, vomiting, heat of surface, rapidity of pulse—than a decided lowering of the body heat, as shown by the thermometer placed in the axilla. But, as a rule, an attack of remittent fever is made up of a series of paroxysms, each of which may be inaugurated by a more or less distinct chill; while yellow fever is essentially a disease of a single paroxysm, in which, as shown by our charts, the acme of temperature is reached early in the attack, and the remission is characterized by a descending line extending often through a period of three or four days. In remittent fever, on the contrary, the fall attending the remission is an abrupt one. The tongue in remittent fever is usually heavily covered with a yellowish or brownish coating, it is broad, and often marked upon the margins by indentations made by the teeth, showing that it is swollen. In yellow fever the tongue is usually narrow and pointed, often tremulous; at the outset of an attack it may be comparatively clean, or it is covered with a thin white, or heavy cottony coating—occasionally yellowish. The yellow color of the skin in bilious remittent fever does not differ from that of simple jaundice, and is very persistent when developed; it is, however, far from being a constant symptom. The same is true of the jaundice which is occasionally developed during convalescence from yellow fever. But, besides this, there is a more or less pronounced yellow tinge of the conjunctivæ and skin in yellow fever, which appears during the latter part of the first, or early in the second, stage, and which in convalescence quickly disappears. In fatal cases this yellow color is more pronounced, and may assume a dingy brown or mahogany color; the dependent portions of the body after death have a livid, mottled appearance, and the discoloration resembles that following a bruise which has produced an effusion of blood. In the malarial fevers the spleen is more or less swollen and tender, while in yellow fever it is not involved. In yellow fever the intellectual faculties are commonly unimpaired, at least during the febrile stage, and often up to the moment of the fatal termination; on the contrary, delirium is quite common in severe attacks of bilious remittent fever. In yellow fever vomiting of bilious matter is rare, and only occurs, if at all, at the outset of the attack; while later, the vomited matter consists of the fluids ingested, or of a transparent acid fluid containing flocculi of mucus, or of one of the varieties of black vomit; in remittent fever the vomited matters are nearly always colored with bile—indeed, bilious vomiting is commonly a marked feature of the disease. In remittent fever, according to La Roche, the quantity of uric acid in the urine is notably increased, being in some cases eight times as great as in health; in yellow fever it is diminished—the amount of urea is diminished in both diseases. Suppression of urine is extremely rare in bilious remittent fever, and extremely common in fatal cases of yellow fever; the uniform presence of albumen in the latter disease has already been noted. Finally, the marked hemorrhagic tendency in yellow fever distinguishes this disease from bilious remittent fever.

There is a form of malarial fever, however, the so-called "hemorrhagic malarial fever," which is characterized by the periodical appearance of blood pigment in the urine, with sometimes a tendency to hemorrhage from mucous surfaces. This is the *fievre bilieuse melanurique* of the French authors. The clinical history of this disease

differs entirely from that of yellow fever. The distinguishing characters are given by Berenger-Feraud as follows:

"Prolonged residence in a malarial country is the most powerful, and, indeed, indispensable predisposing cause.

"The disease is always preceded by numerous attacks of malarial fever, simple at first, then more and more complicated, and taking in general more and more of a bilious aspect, producing a very decided anæmia.

"Icterus appears at the outset of the attack, and is never wanting; it gives from the commencement and throughout the attack a uniform yellow color to the patient, varying from greenish-yellow to a decided yellow ocher.

"The march is intermittent or remittent from the first, and the pulse, urine, and vomiting follow very exactly the variations of temperature. * * *

"The vomiting is bilious, of a decided green color; it is a constant symptom at the outset of an attack, and is arrested with the termination of one attack to reappear with the next.

"After the first, or febrile, period the vomiting continues, but preserves the same characters; it stains linen a bright green, and when collected in a basin it appears transparent, and is of a beautiful emerald-green or olive color.

"The tongue is moist, broad, covered at first with a heavy white fur, which soon receives a greenish tint from the vomited matters. The tongue is not red, either upon its tip or edges; it remains broad, heavily coated, and moist to the end of the malady.

"The urine is black from the commencement, and its color is characteristic, so that the patient himself is struck with it. It is usually abundant and frequently passed, and only has the melanuric aspect during the attack."

In malarial hæmaturia, as seen in this country, the urine varies in color, according to the amount of blood pigment present, from a light red to a deep claret, or porter color. It is usually acid, and always albuminous. The presence of red-blood corpuscles has frequently been demonstrated by microscopical examination, and tube casts, made up of granular matter and red corpuscles, are often present in the urine. In the gravest form of the disease the patient falls into a typhoid condition, and at this time epistaxis is likely to occur, or there may be, in rare cases, hemorrhage from the stomach or bowels.

The differential diagnosis between yellow fever and dengue will depend mainly upon the fatal character of the first-mentioned disease, the presence of albumen in the urine, and the hemorrhagic tendency, as contrasted with the absence of these features and the skin eruptions and arthritic pains in the other. Dengue, like mild yellow fever, often terminates, after a febrile stage of 48 hours' duration, in complete apyrexia, and the patients are able to resume their ordinary occupations, although feeling stiff and sore when making any movement; more frequently there is a return of the fever attended by a rubeoloid eruption, which ends in desquamation, and is accompanied with a return of the arthritic and muscular pains. This stage may last but 3 or 4 days, or may be of much longer duration, but in any case the disease is not attended with danger to life.

The *bilious typhoid* of Griesinger presents some points of resemblance with yellow fever, but the clinical and pathological features are sufficiently distinct to make it improbable that errors in diagnosis will occur when due care is taken.

Quite recently, Dr. Diamantopulos, of Smyrna, has given an account of a disease under the name of *typhus icteroides*, which is said to be distinct from the bilious typhoid of the German authors, and which prevails upon the coast of the mainland, and upon the islands of the Ægean Sea. This name has frequently been applied to yellow fever, which the disease described resembles in many particulars, although no doubt distinct from it. It prevails in the summer and autumn. The attack begins with a chill, followed by fever, intense headache, pain in back and limbs, jactitation, etc. The eyes are described as injected, glistening, and humid; the

epigastrium is tender on pressure, the liver and spleen are more or less enlarged. There is a remission of the fever on the third or fourth day, and the second stage is inaugurated by a general improvement in the appearance and feelings of the patient; later (fifth or sixth day), icterus is developed; this is intense, giving to the whole surface a golden or orange-yellow color. Epistaxis is frequent in this stage, and hemorrhage from the bowels, bloody urine, and petechiæ in certain cases, show that the disease has a hemorrhagic tendency. It is said to prevail endemically, or as an epidemic, and to be miasmatic and not contagious. Perhaps this is the same disease as was epidemic on the island of Mauritius in 1867, an account of which has been given by Pelleran in the *Archives de Medecine navale* (tome 36, 1881). This author, after stating that up to the year 1867, the opinion was generally entertained that malarial fevers did not exist in Mauritius, goes on to say that in 1867 "the effects were terrible and so unusual that the epidemic was ascribed to importation." One of the reporters quoted by Pelleran gives the clinical features as follows: "The patient fell suddenly ill when in perfect health, often after a full meal. The skin became hot, the face red, the eyes injected, the pulse mounted to 90 or 100; the skin became yellow, the tongue dry and glossy, perspiration abundant, accompanied by a cold breath, and exceedingly feeble pulse; afterward followed intense prostration, with intolerable pain in the back, loins, and region of the liver. The poor patient after 20 to 30 hours died in a state of collapse similar to that of cholera. Another character of the fever was its tendency to relapse, to occur at all seasons of the year, in dry, elevated localities as well as in those which were low and damp, and to be accompanied by hemorrhages.

It is evident that these fevers, if they prevailed in the same areas as yellow fever, might give rise to confusion in the diagnosis; and also, that our knowledge of the pathology of tropical fevers is far from being complete.

Many of the physicians practicing in the French Antilles recognize a fever called by them *fievre inflammatoire* as distinct from yellow fever. Berenger-Feraud has shown, however, that it is in fact identical with this disease, and only represents one of its grades of severity.

TREATMENT.

The unsatisfactory results obtained in the various methods of treatment proposed are shown by the fact that a majority of the physicians in those parts of the world where yellow fever prevails, who have had an extended experience, agree that active medication is injurious, and have settled upon an expectant or symptomatic treatment, with careful nursing, as giving the most favorable results. Yet the records of the past show that a certain proportion of the cases recover under all modes of treatment, even the most active and opposed to our present views. The famous Dr. Rush, of Philadelphia, was thoroughly convinced of the advantages of copious bleeding during the first stage of the disease, and ascribed the more favorable results obtained in the epidemic of 1793 as compared with those of 1741, 1747 and 1762 to the more liberal use of the lancet. Venesection was often repeated from five to ten times by Rush and other practitioners of this date, and the amount of blood extracted often exceeded 100 ounces—150 and even 200 in some cases. This excessive bleeding was soon abandoned, but the propriety of drawing blood at the outset of the attack was maintained by many practitioners in the United States and the West Indies for many years after the "heroic" treatment of Rush had been abandoned, and the statistics offered in favor of this practice certainly compare favorably with the results obtained at the present day by expectant treatment. Thus Dr. Catel, who was in charge of a military hospital at Martinique, reports that, out of 176 patients treated during the first 24 hours by bleeding, 5 only died—a mortality of 1 in 35; of 108 treated on the second day, 11 died, or 1 in 9; and of 143 treated on the third nay, 40 died, or 1 in 3. Dr. Lefort, a physician of the same place, also reports that 4 out of 5 of those bled on

the first day recovered, and asserts that on the second day blood-letting affords six times less chance of success (La Roche).

The lancet has been entirely abandoned, in accordance with the changed views as to the pathology of febrile affections, but it may be questioned whether in the plethoric new-comers in tropical regions, who furnish so large a quota to the mortality list from yellow fever, a single bleeding at the outset of the attack might not still be practiced with benefit.

Emetics were formerly considered an essential part of the antiphlogistic treatment. We speak of them only to condemn them, except in those cases in which at the outset of an attack there is evidence that the stomach contains undigested and fermenting material which is the cause of nausea and distress. In this case a simple emetic may be administered, with the sole object of unloading the stomach.

Purgatives have at all times been considered useful at the outset of an attack of yellow fever, and it is the standard treatment, wherever the disease prevails, to administer a cathartic of some kind as soon as the patient comes under observation. Opinions differ as to the best form of cathartic medicine; some prefer a mercurial, some a saline, and others a full dose of oleum ricini. On account of its prompt and thorough action and the absence of irritating properties, the last mentioned medicine is a favorite in our own Southern cities, in the West Indies and in Brazil, especially in domestic practice among the creole population. The dose usually given might seem excessive to those who have not seen its favorable action; half a tumbler full, or even more, is the common dose for an adult—2 to 4 fluid ounces. Many physicians still prefer a mercurial purge, followed, if necessary, by a saline cathartic, and calomel is the mercurial usually selected. This was formerly given by some practitioners in enormous doses—40 to 60 grains—for its "sedative" effect, and by others in repeated small doses as a "salivant." Dr. Rush introduced the use of mercury as a salivant in the treatment of yellow fever in the epidemic of 1793, and "encouraged by the good effects observed on that occasion from a salivation, he was induced the next year to excite it as early as possible in all those cases which did not yield immediately to bleeding and purging. He was delighted to find that it immediately attracted and concentrated in the mouth all the scattered pains of every part of the body, checked nausea and vomiting, and gradually, when it was copious, reduced the pulse, thereby preventing the necessity of further bleeding and purging." He used mercury still more extensively in 1797; and during the memorable epidemic of 1798 a salivation was found by him to be the most certain remedy of any that was used in this fever, for he "did not lose a single patient in whom the mercury acted upon the salivary glands" (La Roche). It is scarcely necessary to remark that this treatment has been entirely abandoned, and when calomel is given to-day it is usually for its cathartic effect, or for its supposed action upon the liver. It may be, however, that it is useful in another way. As we have indicated in discussing the etiology of the disease, it seems extremely probable that it is caused by a microbe whose habitat is in the alimentary canal. If this is in fact the case, the indications are apparent, viz, to keep the *primæ viæ* as nearly empty as possible, so that the multiplication of the specific microörganism may be restricted by want of pabulum; and also for the purpose of removing the microbe and its poisonous products; second, the administration of germicide and antiseptic remedies, with a view to the destruction of the germ, or at least of restricting its development. In carrying out both of these indications it will be necessary to avoid any measures which would induce gastric or intestinal irritation; for, as we have seen, the gastro-intestinal mucous membrane is in a hyperæmic condition early in the attack, and experience has demonstrated that this condition is aggravated by active medication, or by the presence of food—even the simplest—and that the gravest danger may result from the administration of any thing which may add to the irritation of the stomach or intestine, the normal functions of which seem to be arrested as a result of the action of the toxic agent which gives rise to the morbid phenomena. For this reason it is necessary to administer

cathartics with great caution, if they seem called for after the second day of the disease. As a rule, it will be best to spare the stomach, and to move the bowels by purgative enemata.

The prevailing opinion that yellow fever is closely allied to the malarial fevers led many physicians, during the first half of the present century, to prescribe quinine in the expectation that it might prove a specific in this disease. This expectation was not fulfilled, but there are still a certain number of experienced physicians who think a full dose at the outset of an attack to be beneficial; others consider it injurious, and as a rule it has been abandoned. There is, however, much evidence in favor of the practice, although the benefits to be derived from it have no doubt been overstated. Dr. Fenner, in his account of the epidemic of 1849, states that the results obtained in the abortive method of treatment by quinine were entirely successful. The dose given was from 20 to 30 grains, with a moderate quantity of morphine or opium, after having unloaded the bowels with a purgative enema. Dr. Fenner says: "This would generally reduce the vascular and nervous excitement completely in the course of a few hours, throw the patient into a profuse perspiration, relieve all pain, and produce sleep." This is certainly a very desirable result, and while to-day it is generally admitted that quinine has no specific action in yellow fever, and that after the first day or two it is liable to do harm by irritating the stomach, the writer is disposed to think that, as an antipyretic and nervous sedative at the outset of the attack, it may often be administered with advantage. This was the conviction of some of our most experienced army surgeons who encountered the disease at Vera Cruz during the Mexican war, and subsequently in New Orleans.

Dr. Anderson, of Mobile, who, with his associate in practice, treated a large number of cases during the epidemic of 1853, says: "The marked and almost magic effect of a large dose of quinine at the outset was so apparent that they (himself and associate) would have considered it little short of trifling with human life to have adopted any other treatment. They will not deny that there were cases in which it did no good; in fact, in those cases where there was at the commencement decided congestion of the brain, it may sometimes have done harm."

Blair, whose experience was very extensive, and whose treatment seems to have been unusually successful, gave 20 grains of calomel and 25 grains of quinine at the outset of an attack, and under certain circumstances repeated the dose several times.

Dr. Porcher, of Charleston, follows Blair's treatment so far as the first dose is concerned, but protests against a repetition of it. We think he is quite right in this, and that his remarks upon treatment are extremely judicious. In some of the new antipyretics and nervous sedatives we have agents which are probably superior to quinine for reducing the temperature and relieving the nervous symptoms; favorable reports have already been made with reference to some of these. In the epidemic of last year (1887) at Key West, Drs. Porter and Schweringen used *antipyrin*, as they believe, to the advantage of their patients; it has also been prescribed quite extensively in Havana, but I have seen no exact statements as to the results obtained. Nageli, a German physician practicing in Rio Janeiro, has given a very favorable account of results obtained in the use of *kairin*. According to this author, of seven cases treated from the beginning, five of which were severe (three had black vomit), all recovered. The course of the disease was not shortened, but the fever is said to have been controlled. The medicine was administered from the outset in doses of 1 gramme every hour, in capsules. This treatment is said to have quickly reduced the temperature, after three or four doses, to 38° (100.4° F.) or below; at the same time abundant perspiration occurred and the pains were relieved. Doses of one-half a gramme were given every hour after the temperature was reduced to the normal to keep it from again mounting. Iced drinks were given, but no food; after the fourth or fifth day it was sometimes necessary to increase the dose to 1.5 to 2 grammes in order to control the return of fever. The urinary secretion continued during the use of the

remedy, and Nageli thought that less albumen was present than in similar cases not so treated. As the account of this method of treatment was published in 1884, and we have not heard that it has been adopted to any extent in Rio, the supposition is that, like many other methods, it has not stood the test of fuller experience. In the feeble condition of the heart in the second stage of yellow fever, we should anticipate that the large doses administered, as much as 14 grammes in the 24 hours, might be dangerous; but as all of Dr. Nageli's patients are said to have recovered we can not urge this objection.

Many of the French physicians administer *bromide of potassium* as a nervous sedative, but we anticipate that *antipyrin*, or one of the more recent remedies of this class, will be found the most useful in meeting this indication and for the reduction of temperature; there is no reason to expect, however, that they will prove to be specifics—they simply meet certain indications in the symptomatic treatment of the disease. Some of the physicians in Rio Janeiro have prescribed *jaborandi*, and think well of it, without, however, claiming that it has any specific power. When the skin is hot and dry it may serve a useful purpose, but it will be necessary to guard against the depressing effects of the remedy. Recently, Dr. Hebersmith, of the Marine Hospital Service, has given the muriate of pilocarpin in several cases, and he thinks with good results. Doses of one-eighth to one-fourth grain were given hypodermically, and produced profuse perspiration, with reduction of temperature. But as several of his cases were colored persons, and the initial temperature was moderate in all, we are not prepared to admit that recovery was due to the administration of this medicine.

Aconite, digitalis, and veratrine have been tested in the West Indies and elsewhere, in full sedative doses. Feraud says of these medicines that "the attempts, although sufficiently numerous, have not yet furnished any decided results." After a careful trial of digitalis in doses of 2 to 3 grammes (30 to 45 minims) of the tincture per diem, the author quoted arrives at the conclusion that in mild cases it seems to cause the fever to fall more quickly, but in intense cases it had not the same power. His general conclusion is, that aconite, digitalis, and veratrine are superior to quinine, and that in moderate doses they are useful in allaying nervous excitement, and in moderating to a slight extent the fever. The writer has been in the habit of giving small doses of aconite, in combination with a mild diaphoretic, during the febrile stage of yellow fever, and has thought that some benefit was derived from the treatment, although it must be confessed that this has not been very marked.

Dr. Bemiss, of New Orleans, writes of this class of medicines as follows:

"It is the practice of some physicians to attempt the reduction of fever by large injections of cold water, which are said to prove very efficient antipyretics. Aconite, veratrum, tartrate of antimony, and ipecac are frequently exhibited. A cautious use of one or the other of the first two on this list may prove beneficial, but their injudicious or careless exhibition may do irreparable harm. *We have seen digitalis produce unquestionably good effect in mitigating fever, and have often administered it in doses of thirty to sixty drops of the tincture every third or fourth hour.* It is best to give it in solutions of acetate of ammonia or potash."

Dr. Porcher, of Charleston, gives the following mixture as a gentle diuretic and diaphoretic.

℞ Acetate of potash, ℥i
Citrate of potash, ℥i
Morphine, gr. j
Camphor water, ℥ vi

M. A dessert-spoonful to be given every two or three hours as long as there is heat of skin.

Some formula of this kind, which has a gentle sedative and diaphoretic effect, is useful so long as it does not disturb the stomach. Many practitioners refrain from giving any medicine at all, but permit the patients to swallow bits of *ice*, or small

quantities of *ice cold water* at frequent intervals. This we quite approve of, or *iced carbonic acid water* may be given. Others again give *warm drinks* to promote perspiration. This is the standard "creole treatment" in New Orleans, and in many parts of the West Indies. As the mortality among this class is small, they are well satisfied with the results obtained, but many strangers, unacclimatized, who have received the creole treatment, die, in spite of the dose of castor oil, the *hot pediluvia*, and the orange-leaf tea. This treatment, with careful nursing is, however, infinitely superior to the active medication of the novice in the treatment of this treacherous disease, for it is very easy to kill yellow fever patients.

Dr. Pardinas, formerly director of the Military Hospital in Havana, gave full doses of the *tr. ferri chloridi* throughout the disease, and without claiming for it any specific virtues, he was convinced that the mortality was less under its use than in other methods of treatment with which he was familiar. The director of the Jura-juba Hospital, at Rio Janeiro, gave me the following formula, which, after trying many different modes of treatment, he has adopted as being, in his opinion, the best:

℞ Perchloride of iron (normal solution), grammes iv
Tincture of iodine, gramme i
Distilled water, grammes 400

M. A tablespoonful to be given every hour.

Some physicians in the United States have also used the tincture of the chloride of iron in full doses, and have reported favorable results; but it has not come into general use, even in those places from which the favorable reports have come. In Savannah, during the epidemic of 1854, it was prescribed extensively by Drs. Wildman and Harris. The last named physician reported that, out of two or three hundred cases treated in this way, he only lost six. La Roche, in commenting upon this report, says: "Unfortunately the remedy, though so advantageous when administered to the sufferers of Savannah, was powerless in the cases of those to whom it owed its celebrity, for both Dr. Wildman and Dr. Harris fell victims to the prevailing epidemic."

Baths of all kinds—hot, cold, tepid, medicated—have been given in yellow fever, and the evidence is varied as to their utility. The most judicious and experienced practitioners are, however, in accord as to the danger of disturbing the patient to the extent demanded by frequent baths, and, as a rule, content themselves with *hot pediluria* at the outset of the attack, and with sponging the surface with cold water or evaporating lotions to reduce the temperature. Very hot or very cold baths are condemned by Feraud, on the ground that they are likely, even in the first stage of the disease, to produce visceral congestions, to which there is a great tendency in this disease. In the southern part of our own country, and in the West Indies, *a hot mustard foot-bath*, administered at the outset of the attack, is a standard method of treatment. The patient is wrapped in a blanket, and sits with his feet and legs immersed in a bucket of water as hot as he can bear, to which a liberal quantity of mustard has been added. This has a tendency to relieve cerebral congestion and headache, and often produces a free perspiration. It may be repeated several times during the first 24 hours. Cold applications to the head and repeated sponging of the hands, arms, and chest with cold water, give comfort to the patient, and are of decided benefit on account of their antipyretic effect. Some practitioners recommend the use of tepid water for sponging the surface; Feraud prefers a mixture of one part of aromatic alcohol with three parts of water; Dr. Peyre Porcher recommends "the assiduous and protracted applications of ice-cold water to the head, hands, and arms, as long as they are abnormally hot." This practice is justified by the results of his treatment, and we think it entirely safe and extremely useful. The knowledge that exposure to cool currents of air, or by removing the bed-covers at night when the weather has suddenly turned cold, is attended with the greatest danger, on account of the visceral congestions—especially of the kidneys—which result from such

exposure, has led many physicians to refrain from making use of cold lotions to the surface. The same apprehension has also led to an excessive use of blankets, and the poor patient, who is burning up with fever, is sometimes smothered in blankets and carefully guarded to prevent him from exposing hand or foot to the air.

Towards the end of the first stage, and throughout the second stage of the disease, this watchfulness to prevent exposure of the body to cool currents of air is very necessary; indeed, it is important at all times if the external temperature is low, and especially at night, but the object in view can be obtained without loading the patient with blankets. He should be lightly covered, but carefully guarded from exposure if the weather is cool. At the same time the body may be sponged under the blanket, and iced water applied to the head, face, hands, and arms, during the febrile stage.

Berenger-Feraud speaks highly of the use of large enemata of cold water, frequently repeated, as a means of reducing the temperature. He has made extensive use of this mode of treatment, and has never seen any evil results from it.

The use of *sinapisms* and *vesicants* to relieve visceral congestion by revulsion to the surface is approved by experienced physicians everywhere. We prefer sinapisms and believe that, as a rule, they will accomplish all that can be expected from a blister. The feeling of weight, distress, or absolute pain in the epigastric region, attended with nausea, which is largely due to the hyperæmia of the gastric mucous membrane is often notably relieved by the application of a sinapism to the epigastrium. The patient, who has been restless and uncomfortable, will sometimes quickly fall asleep after such an application. In the same way, cerebral congestion and headache may be relieved by revulsives to the extremities, and lumbar pain by the application of a large sinapism to the loins.

The scanty urinary secretion, and often complete suppression, in yellow fever, seems to call for the use of diuretics, but unfortunately experience teaches that the most reliable medicines of this class do but little good. The kidneys are best relieved by those means which promote perspiration, and by revulsants applied to the loins. Complete suppression is rarely, if ever, relieved by any plan of treatment. In cases with a hot, dry skin, and scanty urinary secretion, with lumbar pain, indicating congestion of the kidneys, we should expect benefit from the hypodermic administration of the muriate of pilocarpine.

Stimulants are rarely required before the fourth or fifth day, and must be given at first cautiously and in small doses, so as not to disturb the stomach; later, however, they are often absolutely demanded to sustain the feeble heart. During the second stage of the disease, when the pulsations of the heart often do not exceed forty or fifty to the minute, there is always a tendency to syncope; this is especially manifested during the night or toward morning, and many a patient whose condition seemed satisfactory at the evening visit has been found by his physician dead the next morning. This dangerous period may often be tided over by the use of stimulants. When the stomach is very irritable, a little iced champagne will often answer better than anything else; but perhaps the best form of stimulant is good brandy, given in teaspoonful doses at intervals of half an hour, more or less, according to circumstances. It should be given ice cold. Later, milk punch, English ale, or porter, may be given in liberal quantities, especially to those who are in the habit of using spirits.

Blair, whose opinion in all that relates to this disease is worthy of attention, prefers hock to any other form of stimulant. He says: "Of all cordials the best is Rhenish wine. When of good quality it is retained when everything else is rejected, and it is universally liked by the patients. I have seen the most excellent effects from its use, and have often given it to the extent of two bottles in twenty-four hours. I believe it has saved many lives, and know of no substitute for it."

Medicines administered by the stomach to control vomiting, as a rule, are worse than useless. Minute doses of *morphine*, administered hypodermically, are sometimes

useful in checking gastric irritability, but this is a dangerous remedy in yellow fever, and only very small doses are tolerated. Blair has seen stupor, prostration, and complete narcotism follow the administration of one-tenth of a grain. On account of the danger attending its administration, he thinks that it will be more judicious to place it in the *index expurgatorius* of yellow fever. Bemiss, whose opinion is of equal value, says: "Vomiting should be met with epispastics, blisters, ice, and small doses of opium, in combination with cherry laurel water and sodæ bicarb." An effort should be made to arrest *hæmorrhage* by the use of ergot, the beneficial effects of which are sometimes well marked. As it is not likely to be absorbed by the stomach, and may cause vomiting, it will be best to administer ergotine hypodermically.

During *convalescence* the sulphate of quinine, in moderate doses, or citrate of iron and quinine, or the standard preparations of iron and of strychnine, will often be useful as tonics; and ale or porter or a sound wine in moderate quantities, will be found beneficial.

The *alimentation* in this disease, as in the specific fevers generally, is a matter of prime importance. During the first part of the febrile stage no food is desired, nor is it required. It is best to give nothing in the way of food for the first three or four days of sickness; after this, if the stomach tolerates it, an ounce or two of iced milk or of chicken broth may be given every two or three hours. If the stomach is very irritable, a smaller quantity at shorter intervals may be given, and it will be best to give the milk in combination with lime water. If the stomach will not retain this, or if it gives distress, do not push it further, but support the strength by nutritious enemata. Even when the stomach is quiet and the patient craves food, it will be necessary to give only liquid nourishment for two or three days, and then to allow nothing but the simplest and most digestible forms of solid foods, for there is always danger of a relapse from imprudence in diet, even in the mildest cases.

Before closing this article the writer ventures to refer briefly to a method of treatment which is still too recent to permit of a definite conclusion as to its value.

This was suggested by me during my recent visit to Havana, and is an attempt to formulate a specific treatment in accordance with my present views of the etiology and pathology of the disease. The intensely acid condition of the urine and of the vomited matters, and the fact that I have usually found the contents of the intestine more or less acid, has led me to think that a very decidedly alkaline treatment might be beneficial, and in view of the probability that the specific infectious agent is located in the alimentary canal, I have combined with the antacid selected an antiseptic agent which is known to restrict the development of micro-organisms when present in very minute quantity.

The formula suggested was as follows:

℞ Bicarbonate of soda, grammes x, (150 grains).
Bichloride of mercury, centigrammes i i (3 grain).
Pure water, litre j, (1 quart).

M. Sig. 50 grammes (3 tablespoonfuls) every hour; to be given ice cold.

A letter recently received from my friend, Dr. D. M. Burgess, sanitary inspector at Havana, gives the following account of the results up to the present date. Dr. Burgess says:

"Ten cases (six severe ones) have now been treated at Garcini by your alkaline and bichloride method and all have recovered; none subjected to that method have died. Three were treated successfully at another hospital here. Four are to-day receiving the treatment at Garcini, and about an equal number at each of two other institutions."

This article in Wood's Hand-book of the Medical Sciences was written soon after my return from Cuba, in 1888. Since that time I have received much evidence in favor of the mode of treatment referred to at its close.

The following Preliminary Note upon a New Method of Treating Yellow Fever was published in the Therapeutic Gazette of August 15, 1888:

In view of the recent announcement that yellow fever has reappeared in Florida, and the possibility that it may soon prevail as an epidemic in that State, the writer has decided to report the favorable results recently attained in the treatment of the disease in Havana by a method proposed by him, although the number of cases is still comparatively small.

My recent researches in Havana have led me to think it very probable that in yellow fever, as in cholera, the specific microörganism causing the disease is located in the alimentary canal. While this is not proved, it is demonstrated that, as a rule, no microörganism capable of development in the culture media usually employed by bacteriologists is present in the blood or tissues of those recently dead from yellow fever. This view naturally suggests intestinal antisepsis as a mode of treatment.

It is well known that in yellow fever the urine and the vomited matters are highly acid. I have also found the intestinal contents to have usually a more or less decided acid reaction. A microbe, therefore, capable of multiplying in the stomach and intestine in this disease, must be able to grow in an acid medium. But aside from this theoretical reason for prescribing alkalies, the highly acid condition of the secretions furnishes an indication for such a treatment, and the writer has long desired an opportunity to see a thorough trial of a decidedly alkaline treatment.

These considerations induced me during my recent visit to Havana to propose the following formula:

℞ Sodii bicarb.....grammes x (gr. 150);
Hydrarg. chlorid. corrosiv.....centigrammes ii ($\frac{3}{10}$ gr.)
Aquæ puræ.....litre i (1 qt.)
M. Sig. 50 grammes (about $1\frac{3}{4}$ ounces) every hour; to be given ice cold.

This treatment was adopted by Dr. Raphael Weiss house physician at the Garcini Hospital, and I have just received from him a record of twelve cases treated by the director of the hospital, Dr. Francis Cabera, and himself.

The complete temperature charts sent to me by Dr. Weiss sustain the diagnosis in all of these cases, and this is confirmed by Dr. Daniel M. Burgess, United States sanitary inspector, Marine Hospital Service at Havana, who writes me:

"HAVANA, *July* 18, 1888.

"MY DEAR DR. STERNBERG: I send you by this mail Dr. Weiss's report of twelve cases treated by the alkaline and bichloride method. Although they were all Spaniards, and were not directly under my care, I had sufficient supervision of the cases to satisfy me that they were all genuine yellow fever. It will be seen that they all recovered, and I will add that every case so far treated at the Garcini by that method has recovered. While these twelve cases were being treated, and a little before, eight cases were treated in the same institution by other methods, and five of the eight died."

Dr. Weiss writes me that in some of the more severe cases he increased the amount of bichloride to 5 centigrammes (three-quarters of a grain) per litre. He says:

"Administering to the sick the bichloride of mercury, according to your directions, the gastric phenomena are very much modified; the gastric sediments appear later, and in no case have they been entirely dark. In some cases, truly dangerous on account of the state of the patient in a general sense, gastric sediments" (black vomit) "did not appear, nor even nausea. The pains in the stomach were not so severe."

In some of the cases Dr. Weiss added benzoate of sodium to the formula for its laxative effect, and administered daily two enemas containing each a gramme (15 grains) of the phenate of sodium.

Sherry wine and hypodermic injections of ether were given during the second stage,

when the pulse was feeble and the condition of the patient seemed to call for a stimulant.

With reference to the urinary secretion, Dr. Weiss writes:

"The urine has always been abundant. In some cases the density lowered, but was compensated by a large quantity of urine. Albumen did not appear in some of the cases, in others the amount was small, and in others it reached 2 grammes to the litre. The albumen has always disappeared on the second or third day and then the fever ceased."

Although Dr. Weiss has sent me the clinical records of only twelve cases, he says in his letter:

"I have dealt with thirteen sick from the beginning of the disease and all have recovered. * * * Before using this new treatment, during the present season, we had eight cases which we treated from the first day by the old method" (mainly symptomatic) "and five of the number perished."

This mode of treatment will, of course, require a more extended trial before the results can be fairly compared with those obtained by other methods. I may remark, however, that in similar cases treated by the "expectant" method the mortality rarely falls below 20 per cent., and in hospital practice is often 50 per cent. or even more.

Whether the favorable results are to be ascribed to the alkali, or to the antiseptic action of the bichloride, can only be determined by testing the two remedies separately; but I am very much disposed to ascribe the unusual absence of gastric disturbance and the abundant secretion of urine noted to the alkaline element of the treatment, and it is extremely doubtful whether the bichloride reaches the intestine in sufficient quantity to exercise any decided antiseptic effect in that portion of the alimentary canal. The bicarbonate should be free from carbonate of sodium, as the mercuric chloride is decomposed by this salt and by alkalies generally. It is possible that the biniodide might be substituted to advantage in the formula above given.

In some experiments, made by the writer several years since to determine the comparative antiseptic value of the salts and oxides of mercury, the following results were obtained:

	Active.	Failed.
Biniodide of mercury	1 to 20,000	1 to 40,000
Bichloride of mercury	1 to 15,000	1 to 20,000
Protiodide of mercury	1 to 10,000	1 to 20,000
Yellow oxide of mercury	1 to 1,000	1 to 2,000
Black oxide of mercury	1 to 500	1 to 1,000
Calomel	.....	1 to 100
Blue mass	.....	1 to 100

"In every case the antiseptic was carefully weighed and added to 100 cubic centimetres of beef-peptone solution, or of veal broth. A similar quantity of the culture fluid was put up as a *temoin* without the addition of the antiseptic. As the oxides and iodides of mercury are insoluble in water, the bottle was repeatedly shaken in order to dissolve in the albuminous culture fluid as much of the antiseptic as possible. An undissolved remnant could, however, be recognized at the bottom of the bottle after this repeated shaking. Two drops of broken down beef stock were added to each bottle to cause speedy putrefaction of the culture-fluid in the absence of a sufficiently potent inhibition of the developing power of the bacteria of putrefaction. In every case in the comparative experiment the culture fluid became clouded, and had a putrefactive odor at the end of 24 hours.

"The first column in our table shows the proportion in which the culture fluid was preserved from any appearance of decomposition for at least a week, the duration of

the experiment. In the proportion given in the second column a decided inhibiting power was shown, except in the case of calomel and blue mass, which, in the proportion given (1 to 100), gave no evidence of antiseptic power. The other salts and oxides in the list prevented decomposition for 24 hours in the proportion given in the second column, and it was not until the second day that the bacteria of putrefaction commenced to form a cloud at the upper surface of the fluid, which gradually extended until the fluid had entirely broken down, usually by the third or fourth day. The bottles containing the biniodide (1 to 20,000) and the bichloride (1 to 15,000) have now been standing in the laboratory for 3 weeks, and are as transparent and free from odor as the day they were put up."*

The bichloride, the biniodide, and the oxides of mercury exhibited a decided inhibitory (antiseptic) effect upon the development of the bacteria of putrefaction in the proportion given in the second column, and we may use these figures as a guide in our efforts to secure intestinal antisepsis by means of these agents, although it is of course very uncertain how much of the soluble mercuric salt introduced into the stomach reaches the intestine in an active form—probably very little. In the formula which I gave to the physicians of the Garcini Hospital the amount of bichloride prescribed is really only in the proportion of 1 to 50,000 parts of the solution. But I considered it advisable to commence the treatment with an amount that was entirely safe, and to increase the dose afterwards, if it was found to be prudent and desirable to do so. Dr. Weiss has, in fact, increased the dose in several severe cases to 5 centigrammes per litre—1 to 20,000. As all of these cases recovered we may infer that when largely diluted, as in this formula, and given in small quantities at short intervals, it may be safely administered in these doses; but, as already intimated, I am rather disposed to attribute the favorable course of the disease to the full alkaline treatment than to any antiseptic effect of the bichloride in the intestine. In the stomach, however, I should expect a decided antiseptic effect from the medicine as administered; and perhaps this is, after all, what is most needed.

This preliminary note led to an extended trial of the proposed treatment during the epidemics in Florida and Alabama, which occurred soon after it was published. It was also tested with extremely favorable results during the recent severe epidemic in Rio Janeiro, and during the summer of 1889 has again been put to a practical test in Havana. The results, so far as reported to me, are given below. I quote first from an article published in the Therapeutic Gazette of May 15, 1889:

BICARBONATE OF SODIUM AND BICHLORIDE OF MERCURY IN THE TREATMENT OF YELLOW FEVER.

By Geo. M. Sternberg, *Major and Surgeon, U. S. Army.*

In a "preliminary note," published in the Therapeutic Gazette of August 15, 1888, the writer gave the results of a mode of treatment suggested by himself while in Havana last year. The cases referred to in this note were treated at the Garcini Hospital by Dr. Raphael Weiss, to whom I am indebted for clinical histories of twelve successive cases treated without a single fatal result.

The object of the present paper is to record the results of a more extended trial of the same treatment during the recent epidemics at Decatur, Ala., and at Jacksonville, Fla.

Upon my arrival in Decatur, in the early part of October last, I found that yellow

* From a report of the committee on disinfectants of the American Public Health Association, 1885.

fever of a most malignant type was prevailing, and that the mortality had been very great even under the most favorable conditions as to nursing and surroundings. This is shown by the fact that out of ten physicians practicing in the infected area, nine had yellow fever and five died. The one who escaped, Dr. B. F. Cross, had suffered an attack during a previous epidemic in the same place (1878). Soon after my arrival I called attention to the alkaline and bichloride treatment, and, in view of the very unsatisfactory results which had been obtained by other methods, the physicians remaining on duty, two of whom were recent convalescents, one after another adopted the treatment. Since the termination of the epidemic they have kindly given me a statement of the results obtained, and more or less complete clinical histories of their cases.

The formula originally prescribed by me as published in my preliminary note in the Therapeutic Gazette was adhered to. This is as follows:

℞. Sodii bicarb., grammes x (grs. 150).
Hydrarg. chloridi corrosiv., centigrammes ii ($\frac{3}{10}$ gr.)
Aquæ puræ, litre i (1 quart). M.
Sig.: 50 grammes (about 1¾ ounces) every hour; to be given ice cold.

Results of treatment in Decatur.

Treated by—	Whites.			Colored.		
	M.	F.	Died.	M.	F.	Died.
Dr. B. F. Cross	13	7	2	2	2	0
Dr. E. J. Conyngton	5	3	1	1	2	0
Dr. E. M. Littlejohn	3	.....	0	9	7	0
Dr. W. C. Buckley	1	.....	1	5	4	0
Total	22	10	4	17	15	0

I have excluded from the above tabular statement two cases treated by Dr. Cross, in which the treatment was not commenced at the outset of the attack. One of these, a white male, terminated favorably; the other, a white female, resulted fatally. Dr. Cross remarks, with reference to the last-mentioned case: "She was taken sick on the 29th of September and died November 10; was not put on the Sternberg treatment until the latter part of her illness."

The two fatal cases in the practice of Dr. Cross were: No. 1, Mr. J.; aged about 30; taken sick October 18; died October 23, after suppression of urine and black vomit. No. 2, Mrs. D., a feeble woman of 58 or 60, who had recently lost her husband from the same disease.

The fatal case in Dr. Conyngton's practice was: Captain K., "aged about 50 years; a large, plethoric man, rather a heavy drinker; had been in the habit of drinking whisky for 30 years." This patient had suppression of urine on the fourth day of sickness.

The fatal case treated by Dr. Buckley was: Mr. B., aged 33. "The patient had a severe case of yellow fever, but was doing well, considering the severity of his case, until the morning of the seventh day, when he was found much worse. * * * It was ascertained that the family gave him a hearty meal on the sixth day—the one previous to his becoming worse."

Through the kindness of Dr. Jerome Cochran, State health officer, I have received the following official statement of the number of cases and deaths in the Decatur epidemic:

Whites:		Colored:	
Cases	97	Cases	57
Deaths	30	Deaths	5

This gives us a general mortality of 22.72 per cent., and a mortality among the whites alone of 30.92 per cent.

Reference to the above table will show that in a total of 64 cases treated by the alkaline and bichloride method the mortality was only 6.45 per cent., and that the mortality among the whites, considered separately, was 12.5 per cent.

A just comparison, however, requires that we shall deduct the number treated by the method under investigation from the total number of cases occurring during the epidemic. This leaves us 65 whites with 26 deaths, and 25 colored with 5 deaths treated by other methods; and gives a mortality of 40 per cent. among the white and 20 per cent. among the colored population; whereas under the alkaline and bichloride treatment not a single death occurred out of 32 cases among the colored population—many of the cases were bright mulattoes—and the mortality among the whites was reduced to 12.5 per cent.

RESULTS OF TREATMENT IN JACKSONVILLE, FLA.

My "preliminary note" in the Therapeutic Gazette was brought to the attention of some of the physicians in Jacksonville during the recent epidemic, and I am informed that a number of them employed the treatment referred to, and that it gave general satisfaction to those who adopted it. I have as yet only obtained a detailed report from two physicians, but as these reports include a considerable number of cases treated in the Sand Hills Hospital by Dr. Sollace Mitchell, and in private practice by Dr. A. J. Wakefield, they may be taken as fairly representing the general results obtained by this method of treatment during the Jacksonville epidemic.

Dr. Mitchell writes me as follows:

"JACKSONVILLE, FLA., *September* 9, 1889.

"G. M. STERNBERG, M. D.,

"*Baltimore, Md.:*

"MY DEAR DOCTOR: I send you to-day a list of all cases treated upon the bichloride and alkaline treatment; also those upon the triturated bichloride without the alkali. I confess my surprise at the great difference in mortality. I knew that my results with the alkali were far better than without it, but did not realize the great difference until I made out these lists for you. Some of the ages have been approximated, because the book containing the ages, reception, etc., of many has been lost or mislaid. I treated in all 216 cases. I did not begin the use of the bichloride until I had treated some 35 or 40 by other methods, and when the bichloride was begun only every fourth patient was put upon it, then every other patient, and towards the last almost all patients were put upon it (*i. e.*, the alkaline and bichloride treatment. G. M. S.) Cases were chosen by rotation as they came in, different treatment being used in different wards. The bichloride and alkali gave the best results by all odds.

"Most sincerely, yours,

"SOLLACE MITCHELL."

The lists transmitted by Dr. Mitchell give the name, age, color, sex, and result, with a remark as to the severity of each case.

The list of those treated by the alkaline and bichloride method includes 106 cases, with 5 deaths, a mortality of 4.7 per cent.

Seventy-nine of these cases and all of the deaths were whites, a mortality of 6.3 per cent. Twenty-seven cases were colored, with no deaths.

Of the whites, 73 were males and 6 females. The deaths all occurred among the white males, and the mortality among this class, considered separately, were 6.8 per cent. This is certainly a very unusual result, and brings out in a very striking manner the value of this mode of treatment. Yellow fever is well known to be especially fatal among adult males, and in hospital practice a mortality of less than 25 per cent. among this class of cases is exceptional.

The mortality lists published in the newspapers during the Jacksonville epidemic gave the impression that the epidemic was of an unusually mild character. But it

must be remembered that these lists related to the population generally, and included a very large proportion of the colored race, who remained in the infected city, while a large share of the white population left it upon the outbreak of the epidemic. Now, the mortality from this disease among negroes is always small, and is estimated by Dr. Mitchell not to have been over 2 per cent. in the Jacksonville epidemic. Yet the general mortality, as shown by the daily reports published in the newspapers, was in the vicinity of 10 per cent. Dr. Mitchell estimates the mortality among the white population, considered separately, as from 22 to 25 per cent., but has not yet been able to give me the exact figures. This estimate includes both sexes and all ages, and if adult males were considered separately it would no doubt be considerably more than this. With two or three exceptions the 79 whites included in Dr. Mitchell's list were adults; one only was a child of 8 years; 10 were between 15 and 20 years; 25 from 20 to 30; 29 from 30 to 40; 11 from 40 to 50, and 3 from 50 to 60.

The cases are classified by Dr. Mitchell as to severity as follows: "Mild," 46; "severe," 19; "very severe," 14. It must be remembered that the treatment was commenced at the outset, and the mildness of the attack in those cases classified as "mild" may have been due to some extent to the favorable influence of the medicine administered. Indeed, the general result indicates that such was the fact. It is well known to those physicians who have had an extended experience in the treatment of yellow fever that many cases which appear to be mild at the outset in the end terminate fatally, and especially so in adult males, among whom the tendency to suppression of urine seems greater than in the case of females and children.

One of the principal objects which I had in view in prescribing full doses of bicarbonate of sodium at regular intervals was to neutralize the very acid condition of the urine, in the hope that it might be secreted more abundantly and with less injury to the renal parenchyma. The results attained seem to indicate that such is the case, and that the favorable action of the medicine is largely due to this fact.

Dr. Weiss, in transmitting the clinical notes of twelve successive cases treated in the Garcini Hospital, in Havana, in 1888, without a death, says:

"The urine has always been abundant. In some cases the density was lowered, but was compensated by a large quantity of urine. Albumen did not appear in some of the cases, in others the amount was small, and in others it reached 2 grammes to the litre. The albumen has always disappeared on the second or third day, and then the fever ceased."

Dr. Conyngton, of Decatur, remarks:

"One point of special note was that patients on this treatment in my hands never had any symptoms of suppression of urine, but rather the quantity was increased; while, on the other hand, in patients treated by the expectant plan, with diuretics, the urine was scanty, and those that died (3 in number) died of suppression."

Dr. Sollace Mitchell, in a letter dated February 1, speaks of the "marked effect of the treatment" in diminishing the albumen and preventing in a great measure suppression of urine.

Dr. A. J. Wakefield, of Jacksonville, has given me a list of 89 cases treated by himself during the recent epidemic in that city. Of these cases 75 were white and 14 colored. Five deaths occurred among the whites—a mortality of 6.6 per cent. Thirty-nine of the white cases were males and 36 females; 41 of the cases are classed as severe and 48 as mild. One of the fatal cases is said to have been a consumptive; 1 to have been convalescent and to have died from imprudence; in another the remark is made "bad nursing and imprudence"; another, "unfavorable surroundings."

RESULTS OF TREATMENT AT RIO DE JANEIRO.

My friend Dr. Cleary, an American physician practicing at Rio de Janeiro, in a letter dated December 13, 1888, gives me an account of 4 cases treated by this method; 3 made a good recovery and 1 died. The fatal case assumed a severe form from the outset, and was attended with hemorrhage from the vagina. Black vomit and suppression

of urine occurred on the fourth day. Dr. Cleary thinks that in this case no treatment would have been of any avail, and says, in conclusion: "Should any new cases present themselves to me I shall continue using your method, as I believe in it."

Since the above was written I have received a letter from Dr. Cleary, in which he says:

"In all I have treated 34 cases of yellow fever, all of them genuine, but as about one-half did not present albumen in the urine nor "black vomit" they might be contested, yet they all began with sudden chilliness, then excessive temperature, mostly with dry skin (temperature 39.9° to 40.5°), which lasted from 12 to 72 hours, abating somewhat; tongue whitey-yellowish, skin yellow, great hyperæmia of the thoracic integument, some diminution of the urine, gastric uneasiness, and vomiting.

"In my opinion and that of an experienced Brazilian physician they were undoubted cases of yellow fever. Of these 34 cases I lost only 1—that is the one I wrote you about—and in every case I used your formula steadily throughout."

I have recently received a clipping from the Journal of Commerce of Rio de Janeiro, containing a note from Dr. Rocha Faria, inspector of hygiene, in which the formula prescribed by me is given and strongly recommended. Dr. Faria says:

"Some physicians who during the present epidemic have followed this treatment have obtained magnificent results."

The question arises as to whether the favorable results which have thus far attended this mode of treatment are due to the alkaline element of the treatment, or to the antiseptic element, or to the combined action of the two ingredients.

My principal object in suggesting the formula was to test a decidedly alkaline treatment from the outset of the attack, with a view to relieving the gastric distress and acid vomiting which is a prominent feature in cases treated by the expectant method, and also to render the highly acid urine neutral or slightly alkaline, in the hope that the secretion would be more abundant and the tendency to suppression diminished. The treatment appears to meet these important indications; and, without in any sense being "specific," to save life by preventing those structural changes which give rise to passive hemorrhage from the stomach and suppression of urine—two symptoms which present themselves in a majority of the fatal cases.

Bichloride of mercury in a comparatively small amount was added to the formula, not with the idea that it would to any extent destroy pathogenic microörganisms in the intestine, but as an antiseptic, which might be useful in preventing fermentative changes in the stomach, which would perhaps be favored by the free administration of an alkali. The idea has also occurred to me that the specific germ may possibly find a suitable nidus in the acid secretions of the stomach, and in this case the administration of an antiseptic in combination with an alkali would be the most rational treatment. Still, I have not given much weight to this idea. In my "preliminary note" I say: "I am rather disposed to attribute the favorable course of the disease to the full alkaline treatment than to any antiseptic effect of the bichloride in the intestine. In the stomach, however, I should expect a decided antiseptic effect from the medicine as administered, and perhaps this is, after all, what is most needed." I insist upon this point, because the treatment has frequently been referred to by those who have used it as the "bichloride treatment." From my point of view it is essentially an alkaline treatment, with the addition of a certain amount of the bichloride of mercury as an adjuvant to meet certain indications, but which is not administered with the expectation that, by virtue of its germicide power, it may prove a specific in this disease.

Bichloride of mercury has, however, during the past year been administered in considerably larger doses than I have prescribed in the above formula, with the express purpose of destroying pathogenic microörganisms in the intestine. This treatment was proposed by Dr. Paul Gibier during his stay in Havana last year, and has been fairly tested by Dr. Vincent de la Guardia in his wards in the charity hospital of that city.

The idea that the specific infectious agent of yellow fever may, as in cholera, have its habitat in the alimentary canal, has been forced upon the writer by his failure to find it in the blood and tissues of those suffering from the disease. Dr. Gibier has been led to a similar inference, and has even claimed the discovery of a bacillus which, as we think upon insufficient evidence, he supposes to be the specific infectious agent in question. The treatment proposed by him is based upon this idea, and consists in the free administration of purgatives for the purpose of clearing the pathogenic microbes out of the intestine, and of bichloride of mercury in large doses for its germicide effect. So far as the purgation is concerned, experienced physicians in the yellow-fever zone generally agree that it is important to clear out the bowels with a brisk purge at the outset of the attack; but it is also established by experience that active medication of this kind after the first and second day of an attack is likely to do more harm than good, and, in fact, is a dangerous procedure.

I quote from the paper of Dr. de la Guardia, published in the Cronica Medico-Quirurgica, of Havana, as follows:

"We have been constant in giving the patients the series of purges recommended; the first day a saline cathartic, 45 grammes of sulphate of sodium; the second day, 45 grammes of castor oil in emulsion, with an equal quantity of sirup; and, finally, on the third day, 1 gramme of calomel, to which we added 1 gramme of jalap to make it more active, given in two doses at an interval of one hour. The saline purge was frequently rejected by vomiting, and this also happened sometimes with the castor oil; not so with the calomel, which is generally tolerated. During the period of large evacuations we invariably made use of the mercurial medication; using a draft composed of 5 centigrammes of bichloride of mercury, 30 grammes of brandy, and 120 grammes of sweetened water. Some patients rejected this by vomiting immediately after taking it. The patients were made to take this until the seventh, or, in some cases, the eighth day; at this time the favorable or unfavorable result was decided. During his illness the patient was given a large quantity of liquid in the form of chlorhydric lemonade (2 to 1,000) iced. This was well retained by the stomach. Milk was the only food allowed, generally iced. In some of the most severe cases the purgatives were again commenced. Certain symptoms were treated by special measures, in accordance with general principles. Vomiting, for example, was treated by the application of a blister to the epigastrium, and the patient was allowed to swallow small pieces of ice, Rivier's draft, etc. Hemorrhage was treated with ergot, and adynamia by tonics. The bichloride of mercury has rarely given rise to stomatitis, and when this has happened it has been mild in character, and has been rapidly cured with simple emollient washes or with chlorate of potassium.

"Thirty-seven cases were submitted to the mercurial treatment, all of them typical cases of yellow fever, in St. Margaret's ward of the civil hospital. Of these, 22 recovered and 15 died. This gives a mortality of 40 per cent., which is about the average mortality from this disease in Havana; a little more or less, but rather more.

"It will be seen that the result of our scrupulous investigation up to the present time has been, if not disadvantageous, at least without any advantage to the patients treated."

In a letter from Dr. Sollace Mitchell, from which I have already quoted, he says: "Dr. Gibier claims to have suggested the use of bichloride internally some weeks prior to yourself. He says that he suggested one-tenth grain every hour. But as I experimented with doses from one-twentieth to one-sixtieth, and found that in 6 cases when one-twentieth was used severe cramps and diarrhea followed in from 6 to 18 hours, I doubt very much if a patient could bear one-tenth grain with advantage to himself."

Dr. Mitchell has given me a list of 40 cases treated by the bichloride alone; 32 of these were whites and 8 colored; 9 of the whites died, a mortality of 28 per cent.; whereas the mortality among the same class of patients treated by the alkaline and bichloride method was only 6.3 per cent.

Dr. Mitchell has given the following summary statement of the results obtained by the two methods of treatment:

"One hundred and six cases, bichloride and alkaline treatment, with 5 deaths, and 1 recovery from black vomit; 40 cases bichloride treatment, with 9 deaths and 5 recoveries from black vomit."

In Dr. Mitchell's letter to me I read the sentence last quoted, "and 25 recoveries from black vomit," and it was so published. This large proportion of cases with black vomit led me to infer that the bichloride as administered had a tendency to produce passive hemorrhages from the stomach. But Dr. Mitchell has since informed me that this sentence should read "and 5 recoveries from black vomit." My remarks on this point have therefore no foundation.

I have but a single series of cases to report in which the bicarbonate of sodium was administered alone. These cases were treated at the "Quinta La Purissma Concepcion" by Dr. Duran. The formula used was: Bicarbonate of sodium, 6 grammes; sweetened water, 250 grammes. Of this mixtures 2 tablespoonsful were administered every 2 hours, or about 9 grammes (135 grammes) of the bicarbonate of sodium in the 24 hours. I have no information as to the character of the cases or as to how long the treatment was continued. It often happens in the private hospitals of Havana that the patients, when brought to the hospital, have already been sick for several days, and the mortality is greatly influenced by this fact. In all, 30 cases are reported as having been treated by this solution, with 8 deaths, a mortality of 26.6 per cent. At the same time 11 cases were treated by the "antiseptic method," with 6 deaths, a mortality of 54.5 per cent. The antiseptic administered was biniodide of mercury 2 centigrammes, in 250 centigrammes of sweetened water—two tablespoonsful every 2 hours.

It will be noted that the amount of bicarbonate of sodium in the formula used at the Quinta la Purissima Concepcion was less than in my original prescription. I am disposed to think that it would have been better to increase the dose. This was done by Dr. Sollace Mitchell in the Sand Hills Hospital at Jacksonville, and his results fully justify his judgment in this regard. In making my formula at first, in the absence of any clinical experience in this disease with the special remedies I proposed to use, I felt that it would be wise to keep within the bounds of perfect safety, but Dr. Mitchel has shown that the doses of bicarbonate of sodium may be considerably increased not only with safety, but with excellent results. He says, "The formula that I finally adopted was:

℞ Sodii bicarb., grs. x to lx;
Hyd. chlor. corros., gr. 1-30;
Aquæ puræ, ℥ iv. M.

"Give, ice cold, every hour during the day and every 2 hours at night."

This, taking the minimum amount, 10 grains, would make 3 drams of bicarbonate of sodium and six-tenths of a grain of bichloride of mercury in the 24 hours. I prefer to prescribe a sufficient quantity for the 24 hours and to give the medicines more largely diluted. I noticed at Decatur that the patients not only took the mixture as originally prescribed by me without objection, but that they looked forward to the time when the medicine was to be administered, as they found the ice-cold drink refreshing. And as vomiting has not, in my observation, been induced, but on the other hand, gastric irritability has been allayed, I can see no objection to giving the hourly dose in 1½ to 2 fluid ounces of water. Indeed, I think that the taking of a considerable amount of fluid, if it is promptly absorbed by the stomach, is rather beneficial than otherwise. My original prescription contained an amount of water, 1 litre, which in my judgment the patient might be permitted to drink in divided doses in the course of the 24 hours. Under certain circumstances, of course, this might be increased by giving small draughts of cold water or bits of ice in the intervals between the administration of the medicine. But excess in this direction will often induce vomiting, and I have desired to avail myself of the advantage of a

formula which contains a proper amount of water, to be administered at short intervals, instead of leaving the administration of this fluid to the judgment of the nurse, or the desires of the patient. In this way, also, the whole treatment becomes extremely simple and is easily carried out, no small advantage for the busy practitioner during the prevalence of an epidemic.

In view of these considerations and of the experience of Dr. Sollace Mitchell with increased doses of the two ingredients contained in my original formula, I would suggest for further trial the following:

℞ Sodii bicarb., grammes xvi (℥ iv).
Hyd. chlor. corros., centigrammes iii (gr. ½).
Aquæ Puræ, litre 1 (1 quart). M.

Sig: 50 grammes (about 1¾ ounces) every hour; to be given ice cold.

Naturally the amount given should vary according to the age of the patient, and perhaps, also, with reference to the severity of the case. Thus a mild case might have two tablespoonfuls every hour during the day and every two hours at night, while a case, which at the outset appeared to be of a grave character, might take 2 fluid ounces of the mixture every hour day and night. Experience may show that the dose of bicarbonate of sodium may be still further increased in severe cases with benefit to the patient, but I doubt, in view of the evidence above recorded, whether there would be any advantage in increasing the dose of mercuric chloride. It would be a mistake to attempt to substitute a potassium salt for the bicarbonate of sodium in the above formula. The mercuric chloride, which remains in solution in presence of sodium bicarbonate in the proportions prescribed, would be precipitated by potassium carbonate. Moreover, the potassium salts are directly contraindicated in a disease in which there is a great tendency to suppression of urine, and uræmic poisoning. The experiments of Zelz and Ritter, and of Bouchard, show that in uræmia the toxic symptoms are largely due to the retention of potassium salts rather than to urea. Bouchard especially, as a result of his extended experiments, insists upon "the great importance of limiting potassium salts, both in food and medicine, in the treatment of uræmia."

I may add that a rather extended trial of carbonate of potassium in full doses, made by one of the physicians of Decatur during the recent epidemic, was attended with a high rate of mortality, and that the physician referred to himself fell a victim to the disease, although his case at the outset appeared to be rather a mild one. How far the treatment influenced the result it is, of course, impossible to determine, but suppression of urine and black vomit occurred on the fourth day, and death quickly followed.

HAVANNA, *April* 6, 1889.

During the past summer (1889), Dr. D. M. Burgess, United States sanitary inspector at Havana, has treated all of his yellow-fever cases with the alkaline and bichloride method. He reports as follows in a letter dated Havana, December 29, 1889:

I have to report that I have had this season up to date 25 private cases of yellow fever; they were all given "Sternberg's treatment," and while many of them were severe cases, all recovered.

The results of the trial of this treatment, made in the Charity Hospital at Havana by Dr. Vincente de la Guardia and Dr. Emilio Martinez during the past summer, are given in a paper by the last-named gentleman, published in the "Revista de Ciencias Medicas," of September 5, 1889. This report is especially valuable because Dr. La Guardia has had an extended experience in the same hospital with similar cases occurring during preceding years, and because he had

made in the summer of 1888 a careful test of the method of treatment proposed by Dr. Paul Gibier, which consisted in the administration of purgatives, and of full doses of mercuric chloride. His report, which was unfavorable to this mode of treatment, is referred to in my last paper in the Therapeutic Gazette, quoted above.

Dr. Martinez says in the paper referred to:

Associated with Dr. D. Vincente de la Guardia, physician to the Mercedes Hospital, and authorized by the director of this institution, it was resolved to submit the alkaline and mercurial treatment of Dr. Sternberg, which had given such excellent results in the recent epidemic at Decatur, in the United States, to an extensive trial.

* * * * * * *

The plan followed, in general terms was as follows: Upon the admission of the patient a purgative was ordered, if he had not passed the third day, in which case we prescribed a purgative enema. From the first we commenced to administer the following formula:

℞ Bicarbonate of soda, 16 grammes.
Bichloride of mercury, 2 centigrammes.
Water, 1 litre.

Forty-five grammes to be given ice cold every hour, day and night.

For the first 5 or 6 days no food was given. When defervescence commenced we substituted for the potion water containing bicarbonate of soda, 4 parts to 1,000 as an ordinary drink (1 to 2 litres).

Nausea and vomiting was combatted with ice.

We present below the clinical notes so that an exact judgment may be formed as to the value of this method of treatment. It must be remembered that the statistics of hospitals give an excessive mortality not only on account of the gravity of the cases received, but also because of the advanced period of sickness at the time of admission.

We group our cases in three classes: *light cases*, those which entered upon convalescence at the termination of the first period; *common cases*, those which passed to the second period; and *grave cases*, those which present some symptom of gravity.

Clinical notes of 44 cases are given by the author of the paper; of these, 11 are placed in the first category, light cases; 14 in the second, common cases, and 19 in the third, grave cases.

The results are summed up as follows:

Treated	44
Recovered	37
Died	7
Mortality	15.9 per cent.

The official statistics of yellow fever in the Mercedes Hospital in previous years is as follows:

Year.	Treated.	Recovered.	Died.	Mortality.
				Per cent.
1882	187	124	63	33.6
1883	178	103	75	42.1
1884	132	77	55	41.6
1885	40	16	24	60.0
1886	28	11	17	60.7
1887	75	33	42	56.0
1888	72	38	34	47.2
	712	402	310	43.5

Thanks to the courtesy of our confréres in the hospital we have been able to submit to treatment all the cases admitted, but we have excluded two—one which entered on the seventh day with suppression of urine and black vomit, who resisted all treatment; the other entered 6 hours before death and was not seen by us. From the comparison made, it appears that the Sternberg treatment has diminished the mortality to less than one-half the mean mortality of this hospital.

We have observed the following facts: The patients have offered a notable gastric tolerance during the medication; when treated from the first day vomiting has rarely occurred.

The secretion of urine has always been considerable; even in the grave cases, when death occurred, they did not die anuric (La Guardia). Anuria only occurred in an evident manner in a single case (case 28).

From the eighth to the tenth day it is necessary to suspend the bicarbonate, to give stimulants and to combat hemorrhages, adynamia, etc., by the usual means.

REPORT.

The investigations to which this report relates were made in the city of Havana in the summers of 1888 and 1889; in the city of Decatur, Ala., in the autumn of 1888, and in the laboratories of the Johns Hopkins University, where I have continued my researches during the intervals between my visits to the infected localities, and since my return from Havana, in September, 1889, up to the present date.

I.—MATERIAL.

My bacteriological studies have been made with material obtained from forty-three yellow fever cadavers; from "black vomit" and feces of patients in various stages of the disease; and, for comparison, from eighteen cadavers in which death occurred from some other disease than yellow fever, and from feces of healthy individuals.

The autopsies which have furnished me this material are as follows:

No. 1. *Havana, May* 12, 1888.—Soldier in Military Hospital; sick 8 days; black vomit; albuminous urine; autopsy 3½ hours after death. Collected pericardial fluid, blood from heart, bile from gall bladder, urine from bladder, and material from liver, kidney, stomach, and intestine.

No. 2. *Havana, May* 17, 1888.—Attendant in Military Hospital; aged 30; 4 months in Cuba; sick 3 days; autopsy 2 hours after death. Collected material from liver, spleen, kidney, intestine, and stomach, blood from heart, urine from bladder, and fluid from pericardium.

No. 3. *Havana, May* 19, 1888.—Soldier in Military Hospital; sick 6 days; autopsy 7 hours after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, urine from bladder, and pericardial fluid.

No. 4. *Havana, May* 22, 1888.—Soldier in Military Hospital; sick 5 days. Collected material from kidney, spleen, stomach, and intestine, blood from heart, and urine from bladder.

No. 5. *Havana, May* 23, 1888.—Soldier in Military Hospital; sick 6 days; autopsy 4 hours after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder.

No. 6. *Havana, May* 23, 1888.—Soldier in Military Hospital; autopsy 4 hours after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder.

No. 7. *Havana, May* 26, 1888.—Soldier in Military Hospital; sick 4 days; autopsy 2½ hours after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder.

No. 8. *Havana, May* 26, 1888.—Soldier in Military Hospital; sick 6 days; autopsy 2 hours after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder.

No. 9. *Havana, June* 3, 1888.—Soldier in Military Hospital; sick 5 days; autopsy 5 hours after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder.

No. 10. *Havana, June* 6, 1888.—Soldier in Military Hospital; sick 5 days; autopsy 1 hour and 40 minutes after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder.

No. 11. *Decatur, Ala., October* 3, 1888.—Male, aged 35 years; sick 3 days; autopsy 1 hour after death. Collected material from liver, kidney, stomach, and intestine.

No. 12. *Decatur, Ala., October* 5, 1888.—Male, aged 35 years; autopsy 1½ hours after death. Collected material from liver, kidney, stomach, and intestine.

No. 13. *Decatur, Ala., October* 8, 1888.—Male, aged 40; sick 5 days; autopsy 2 hours after death. Collected material from kidney, liver, stomach, and intestine.

No. 14. *Havana, April* 23, 1889.—Soldier in Military Hospital; sick 5 days; autopsy 9 hours after death. Collected material from spleen, liver, kidney, stomach, and intestine, blood from heart, and urine from bladder.

No. 15. *Havana, April* 28, 1889.—Soldier in Military Hospital; sick 10 days; autopsy 9 hours after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder.

No. 16. *Havana, May* 5, 1889.—Soldier in Military Hospital; sick 7 days; autopsy 13½ hours after death. Collected material from liver, kidney, stomach, and intestine.

No. 17. *Havana, May* 12, 1889.—Soldier in Military Hospital; sick 5 days; autopsy 5 hours after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder.

No. 18. *Havana, May* 13, 1889.—Patient in Civil Hospital; male, aged 28 years; sick 5 days; autopsy 2 hours after death. Collected material from liver, kidney, stomach, and intestine, and blood from heart.

No. 19. *Havana, May* 22, 1889.—Soldier in Military Hospital; sick 5 days; autopsy 6 hours after death. Collected material from liver, stomach, and intestine, and blood from heart.

No. 20. *Havana, May* 26, 1889.—Patient in Civil Hospital; autopsy 9 hours after death. Collected material from liver, stomach, and intestine.

No. 21. *Havana, May* 27, 1889.—Soldier in Military Hospital; sick 7 days; autopsy 10 hours after death. Collected material from liver and intestine.

No. 22. *Havana, June* 4, 1889.—Soldier in Military Hospital; sick 5 days. Collected material from liver, stomach, and intestine.

No. 23. *Havana, June* 4, 1889.—Soldier in Military Hospital; sick 8 days; autopsy 10 hours after death. Collected material from liver, stomach, and intestine.

No. 24. *Havana, June* 13, 1889.—Soldier in Military Hospital; sick 5 days; autopsy 4½ hours after death. Collected material from liver, kidney, stomach, and intestine.

No. 25. *Havana, June* 29, 1889.—Soldier in Military Hospital; sick 9 days; autopsy 10½ hours after death. Collected material from liver, stomach, and intestine.

No. 26. *Havana, July* 1, 1889.—Soldier in Military Hospital; sick 9 days, autopsy 5 hours after death. *Doubtful case;* the diagnosis is not supported by the pathological appearances. *Excluded.*

No. 27. *Havana July* 3, 1889.—Patient in Civil Hospital; sick 7 days; autopsy 1 hour and 15 minutes after death. Collected material from liver, stomach, and intestine.

No. 28. *Havana, July* 15, 1889.—Soldier in Military Hospital; autopsy 6 hours after death. Collected material from liver, stomach, and intestine.

No. 29. *Havana, July* 29, 1889.—Soldier in Military Hospital; sick 6 days; autopsy 5 hours after death. Collected material from liver, stomach, and intestine.

No. 30. *Havana, August* 9, 1889.—Soldier in Military Hospital; sick 7 days; autopsy 7½ hours after death. Collected material from liver, stomach, and intestine.

No. 31. *Havana, August* 10, 1889.—Patient in Civil Hospital, male, aged 41; sick 6 days; autopsy 6 hours after death. Collected material from liver, stomach, and intestine.

No. 32. *Havana, August* 12, 1889.—Soldier in Military Hospital; sick 5 days; autopsy 6 hours after death. Collected material from liver, stomach, and intestine.

No. 33. *Havana, August* 13, 1889.—Soldier in Military Hospital; sick 7 days; autopsy 7 hours after death. Collected material from liver, stomach, and intestine.

No. 34. *Havana, August* 13, 1889.—Soldier in Military Hospital; sick 9 days; autopsy 3 hours after death. Collected material from liver.

No. 35. *Havana, August* 15, 1889.—Soldier in Military Hospital; sick 5 days; autopsy 5 hours after death. Collected material from liver and intestine.

No. 36. *Havana, August* 19, 1889.—Soldier in Military Hospital; sick 6 days; autopsy 5 hours after death. Collected material from liver and intestine.

No. 37. *Havana, August* 21, 1889.—Soldier in Military Hospital; sick 4 days; autopsy 6 hours after death. Collected material from liver and intestine.

No. 38. *Havana, August* 22, 1889.—Soldier in Military Hospital; sick 5 days; autopsy 6½ hours after death. Collected material from liver and intestine.

No. 39. *Havana, August* 24, 1889.—Patient in Civil Hospital; male, aged 23; sick 10 days; autopsy 2 hours after death. Collected material from liver.

No. 40. *Havana, August* 24, 1889.—Soldier in Military Hospital; sick 4 days; autopsy 6 hours after death. Collected material from liver.

No. 41. *Havana, August* 26, 1889.—Patient in Civil Hospital; sick 7 days; autopsy 8 hours after death. Collected material from liver.

No. 42. *Havana, August* 26, 1889.—Patient in Civil Hospital; autopsy 4 hours after death. Collected material from liver.

No. 43. *Havana, August* 26, 1889.—Soldier in Military Hospital; sick 7 days; autopsy 6 hours after death. Collected material from liver.

COMPARATIVE AUTOPSIES.

No. 1. *Havana, May* 17, 1889.—Case of tuberculosis in Civil Hospital; autopsy 2 hours after death. Collected material from liver and kidney.

No. 2. *Havana, May* 19, 1889.—Case of tuberculosis in Civil Hospital; autopsy 1¾ hours after death. Collected material from liver and kidney.

No. 3. *Havana, May* 22, 1889.—Case of heart disease in Civil Hospital. Collected material from liver.

No. 4. *Havana, May* 25, 1889.—Case of abscess of liver in Civil Hospital; autopsy 1½ hours after death. Collected material from liver.

No. 5. *Havana, May* 25, 1889.—Insane woman in civil hospital. Brain disease; autopsy 5 hours after death. Collected material from liver.

No. 6. *Havana, May* 30, 1889.—Case of tuberculosis in civil hospital; autopsy 6 hours after death. Collected material from liver.

No. 7. *Havana, June* 2, 1889.—Case of heart disease in civil hospital; autopsy 5 hours after death. Collected material from liver.

No. 8. *Baltimore, October* 30, 1889.—Case of tuberculosis in Johns Hopkins Hospital; autopsy 8 hours after death. Collected material from liver.

No. 9. *Baltimore, November* 12, 1889.—Case of tuberculosis in Johns Hopkins Hospital; autopsy 6½ hours after death. Collected material from liver.

No. 10. *Baltimore, November* 18, 1889.—Case of osteomyelitis of tibia with amyloid liver and kidney. Collected material from liver.

No. 11. *Baltimore, November* 23, 1889.—Case of tuberculosis in Johns Hopkins Hospital; autopsy 24 hours after death. Collected material from liver.

No. 12. *Baltimore, November* 25, 1889.—Case of tuberculosis in Johns Hopkins Hospital; autopsy 8 hours after death. Collected material from liver.

No. 13. *Baltimore, November* 30, 1889.—Death from chloroform; autopsy at once by Dr. Keirle, of Baltimore. Collected material from liver.

No. 14. *Baltimore, November* 30, 1889.—Case of heart disease in Johns Hopkins Hospital, Baltimore; autopsy 7 hours after death. Collected material from liver.

No. 15. *Baltimore, January* 2, 1890.—Peritonitis following laparotomy for ovarian tumor. Case in Johns Hopkins Hospital. Collected material from liver.

No. 16. *Baltimore, January* 6, 1890.—Death in 10 minutes after tapping of abdominal cavity. Case of fibro-cystic tumor of uterus in Johns Hopkins Hospital; autopsy 4 hours after death. Collected material from liver.

No. 17. *Baltimore, January* 13, 1890.—Case of heart disease, Johns Hopkins Hospital; autopsy 4 hours after death. Collected material from liver.

No. 18. *Baltimore, January* 14, 1890.—Pneumonia. Case in Johns Hopkins Hospital; autopsy 24 hours after death. Collected material from liver.

MATERIAL COLLECTED FROM YELLOW-FEVER CASES DURING LIFE.

Havana, May 6, 1888.—Collected black vomit and melanotic discharge from bowels. Case in military hospital, fifth day of disease.

Havana, May 5, 1888.—Cultures made from surface of body of cases in civil hospital

No. 1. Yellow fever convalescent.
No. 2. Case of rheumatism.
No. 3. Case of typhoid fever.
No. 4. Attendant in hospital.
No. 5. Case of yellow fever with black vomit, fifth day of sickness.
No. 6. Dr. Finlay.

Havana, May 29, 1888.—Collected vomit from yellow fever cases in military hospital:

No. 1. Sick 3 days; vomit transparent; acid.
No. 2. Sick 2 days; vomit transparent; acid.
No. 3. Sick 2 days; vomit transparent; acid.

Havana, June 4, 1888.—Collected vomit from patient in Garcini Hospital; sick 3 days; vomit transparent; alkaline.

June 5.—Collected "coffee ground" vomit from same case; acid.

Havana, June 4, 1888.—Collected vomit from case in Garcini Hospital; transparent; acid.

Havana, July 5, 1889.—Collected typical black vomit from case in civil hospital; sick 7 days.

SPECIMENS OF FECES COLLECTED AND EXAMINED IN DECATUR, ALA. (1888), AND IN HAVANA (1889).

Case 1. *October* 3.—Sick 48 hours; thin, yellow color; neutral reaction.

Case 2. *October* 6.—Sick 60 hours (fatal case); thin, yellow color; neutral reaction.

Case 3. *October* 6.—Sick 72 hours (fatal case); mucus, without color; neutral reaction.

Case 4. *October* 7.—Sick 12 hours (fatal case); reaction neutral.

Case 5. *October* 8.—Sick 36 hours; thin, pale yellow; neutral reaction.

Case 6. *October* 9.—Sick 48 hours; thin, pale yellow; neutral reaction.

Case 7. *October* 9.—Sick 4 hours; thin, pale yellow; slightly acid.

Case 8. *October* 10.—Sick 24 hours (fatal case); thin, light yellow color; slightly acid reaction.

Case 9. *October* 10.—Sick 24 hours; thin, light-yellow color; neutral.

Case 10. *October* 14.—Sick 3 days (fatal case); thin, pale yellow color; neutral.

Case 11. *October* 15.—Sick 12 hours.

Case 12. *October* 15.—Sick 2 days (fatal case); thin, pea-soup color; neutral.

Case 13. *October* 16.—Sick 3 days; thin, brown color; slightly acid.

Case 14. *October* 16.—Sick 2 days.

Case 15. *October* 17.—Sick 36 hours (fatal case); very thin, bright yellow color; neutral.
Case 16. *October* 18.—Sick 50 hours (fatal case); thin, clay-colored; slightly acid.
Case 17. *October* 19.—Sick 3 days (fatal case); thin, dark-brown color.
Case 18. *October* 23.—Sick 48 hours; very thin, pale yellow; neutral reaction.
Case 19. *October* 23.—Sick 2 days; thin, pale yellow color; neutral.
Case 20. *October* 25.—Sick 48 hours; thin, brown color; alkaline reaction.
Case 21. *October* 27.—Sick 36 hours (fatal case); thin, color of pea soup; neutral. Same case; third day; thin, light yellow; neutral.
Case 22. *October* 28.—Sick 24 hours; thin, color of pea soup; neutral.
Case 23. *October* 30.—Sick 24 hours; very thin, pale yellow; neutral.
Case 24.—Sick 3 days.
Case 25.—Sick 24 hours.
Case 26.—Sick 5 days.
Case 27.—Sick 48 hours.
Case 28.—Sick 30 hours.
Case 29.—Sick 48 hours.
Case 30.—Sick 36 hours.
Case 31.—Sick 36 hours.
Case 32.—Sick 36 hours.
Case 33.—Sick 48 hours.
Case 34.—Sick 24 hours.
Case 35.—Sick 24 hours.

The material from cases 25 to 36, inclusive, was collected in Decatur by my assistant, Dr. Littlejohn, and sent to me at Baltimore, where the bacteriological examination was made.

At my request, Dr. Littlejohn also sent me feces from fifteen healthy persons and convalescents, for comparison.

Case 37. *Havana, July* 1, 1889.—Collected feces from case in civil hospital; sick 7 days; very low, a milky looking fluid, with acid reaction.
Case 38. *Havana, July* 2, 1889.—Collected feces from case on board German brig; sick 5 days; thin, light yellow, alkaline.
Case 39. *Havana, July* 4, 1889.—Collected feces from case in civil hospital; sick 5 days; thin, yellow color, alkaline.

NOTE.—The alkaline reaction of the feces in many of these cases was no doubt due to the fact that they were on an alkaline treatment.

II.—METHOD OF COLLECTING MATERIAL.

I am in the habit of using as collecting tubes the glass bulbs with a long neck, described by me in a paper read before the American Association for the Advancement of Science in August 1881. The form is shown in Fig. 1.

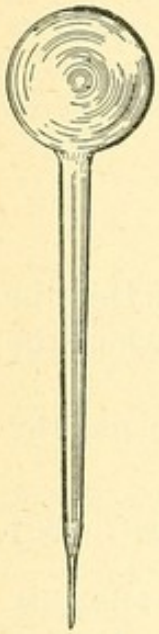


Fig. 1.

These are thoroughly sterilized and hermetically sealed when made. I always carry a large supply of them "into the field" with me, as they serve for various purposes, as will hereafter be shown.

The *modus operandi* of collecting material at an autopsy, as usually practiced by me, is as follows: I expose the abdominal viscera by making an incision from the superior spinous process of the illium on one side, in a line parallel with the long axis of the body up to the margin of the ribs, then across to a corresponding point on the opposite side and down to the other superior spinous process. The large, apron-like flap consisting of the entire anterior wall of the abdominal cavity is thrown back, and gives free access to the viscera contained in this cavity.

It is best to collect from the solid viscera first. The liver is drawn down a little with a tenaculum or forceps and a hot spatula is applied to a point upon its surface. This insures the destruction of any microörganisms which may have fallen upon the surface, or which might be in the cavity of the abdomen before opening it. The spatula is held in position by an assistant until I am ready to introduce the collecting tube. The long stem of this is passed through the flame of an alcohol lamp to sterilize the exterior, the bulb is heated to expand the contained air, and the sealed extremity of the tube is then broken off with sterilized forceps. The preliminary heating of the bulb is to prevent a sudden in-rush of air, by which atmospheric organisms might occasionally gain access to the bulb. The extremity of the stem is then passed into the organ at the point where the hot spatula had been applied.

The air in the bulb being somewhat rarefied by the heat applied, there is a suction force as it cools, and blood from the organ is drawn into the tube.

In order to obtain at the same time crushed tissue, I move the tube backwards and forwards so as to lacerate and crush the parenchyma

of the organ with its broken extremity. By this means I am able to obtain from the liver, the spleen, or the kidney a considerable quantity of crushed parechyma mixed with blood without any possible contamination by microörganisms from without. As soon as the collection is made the extremity of the tube is sealed in an alcohol lamp. Material from the hollow viscera is obtained in the same way. The hot spatula is applied to a convenient point on the walls, and the broken end of the sterilized tube is forced through at this point. My collections of urine through the walls of the bladder, of material from the stomach and intestine, and of blood from the heart have all been made in this way. I am in the habit of making a separate opening through the chest walls over the heart, as I consider it a safer method than to reach the heart by cutting through the diaphragm.

Pieces of tissue for future histological study are cut into small fragments and at once placed in strong alcohol or in Mueller's fluid.

III.—METHODS OF RESEARCH.

The following are the principal methods of research employed.

(*a*) THE DIRECT EXAMINATION OF "SMEAR PREPARATIONS" FROM THE BLOOD AND TISSUES FOR MICROÖRGANISMS.

I have made this examination in the entire series of cases in which I have made autopsies. Usually I have stained these preparations with an aqueous solution of fuchsin, or with Loeffler's solution of methyline blue. I prefer the fuchsin solution because it stains very promptly all of the bacteria with which I am acquainted; and I feel very confident that with my one-eighteenth inch hom. oil im. objective of Zeiss, the Abbe condenser, and a fuchsin-stained "smear preparation" from the blood, liver, or kidney, any microörganism of this class which might be present should be easily seen.

(*b*) AËROBIC CULTURES IN FLESH-PEPTONE-GELATINE, IN AGAR-AGAR JELLY, ETC.

In the whole series of cases I have introduced some of the material collected as heretofore described into one or the other of these media, and often in both. Immediately upon my return to the laboratory after making an autopsy I am in the habit of putting up Esmarch roll-tubes from the material collected, and these are kept under observation, at a proper temperature, for several days at least. In tube No. 1 of a series of three Esmarch roll-tubes I commonly introduce two or three drops of blood, or of crushed liver tissue, etc., so that any microörganism capable of growing in the culture medium employed would be revealed, by the development of colonies, even if present in very small numbers.

In Cuba, during the months of March and April I kept an incubating oven running at a temperature of 35° to 37° C., but later, during the epidemic season, I took it for granted that artificial heat would not be necessary to insure the development of the particular germ I was in search of. Of course our usual medium for plate cultures—flesh-peptone-gelatine containing 10 per cent. of gelatine—was not available in Havana during the summer months without resorting to the use of a refrigerator. I found, however, that when the medium contained 20 per cent. of gelatine it would stand a temperature of 28° C. (82.4° F.), and I was able to use it without a refrigerator during the months of May and June. Later a refrigerator was required even for the 20 per cent. gelatine medium. This contained a chamber which I was able to maintain at a temperature of about 27° C.

So far as I could see, this 20 per cent. gelatine answered quite as well, as a culture medium, as that made in accordance with Koch's standard formula, which contains 10 per cent. of gelatine. Liquefying organisms caused liquefaction as usual, and non-liquefying organisms formed colonies in Esmarch roll-tubes, and grew freely in "stick cultures." But the advantages of an agar medium were apparent, and I accordingly used this very extensively for my roll-tubes, and especially with the addition of 5 per cent. of glycerine. Many of the microörganisms which I encountered and shall hereafter describe, grew luxuriantly in this medium. I also made cultures in an agar or gelatine medium containing 0.2 per cent. of hydrochloric acid. Quite a number of the bacilli which I have isolated grew freely in this acid medium.

I have also tested the growth of the various organisms studied by me upon potato; and for this purpose have used the cylindrical pieces of potato, cut with a slanting surface and sterilized in an ordinary test tube as first recommended by Meade Bolton.

Cultures have also been made in various other media, such as blood serum, veal broth, and *agua coco*. The last-mentioned liquid I have used extensively and find that for a large class of microörganisms it constitutes a very favorable nutrient medium.

(*c*) ANAËROBIC CULTURES.

I made no anaërobic cultures in 1888, but the following year did so in a considerable number of cases. In these I used for the most part agar jelly with 5 per cent. of glycerine.

The blood or liver tissue from one of my collecting tubes was introduced into the liquefied medium kept at a proper temperature in a warm bath (40° C.), and a current of purified hydrogen allowed to pass through it long enough to insure the exclusion of oxygen—usually half an hour or more. My method is as follows:

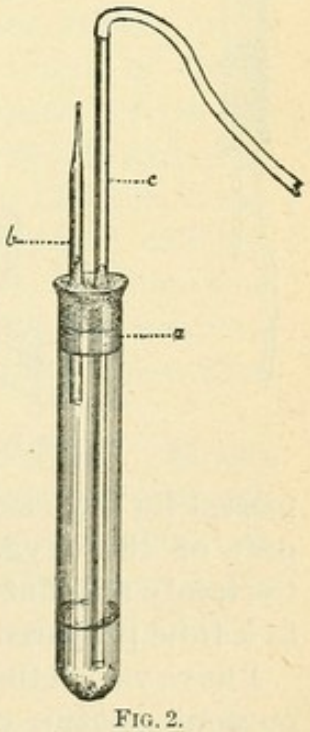


FIG. 2.

Soft rubber corks having two perforations for the passage of glass tubes are cut into sections; these are placed for sometime in 1:1000 solution of mercuric chloride to sterilize them, and then, in a wide-mouthed bottle, having a ground-glass stopper, in strong alcohol. When one of these is to be used it is taken from the alcohol with sterilized forceps and the alcohol is removed by burning. A long and a short glass tube are passed through this rubber stopper, and removing the cotton plug from a test tube in which the anaërobic culture is to be made it is placed as in Fig. 2 (*a*). The long tube, previously sterilized by heat, must pass nearly to the bottom of the liquefied agar. A space of half an inch or more is left above the rubber

stopper, and this I fill with melted sealing wax. I have found by experience that the rubber stopper alone can not be relied upon to retain the hydrogen, no matter how accurately it fits the test tube. Nor have I had good success in the use of melted paraffine. This has a tendency to retract from the glass walls of the test tube, and in practice I find that as a rule the hydrogen soon escapes from a tube closed in this way. It will be seen that my rubber stopper serves chiefly to support the glass tubes, and the melted sealing wax, upon which I depend for the complete sealing up of the mouth of the test tube. The sections of rubber stoppers are of various diameters, and it is easy to select one which will fit the test tube employed.

The long glass tube is now connected with the hydrogen apparatus and the test tube is left in the warm bath to keep the agar jelly from becoming solid. At the end of half an hour the outlet tube *b* is sealed in the flame of an alcohol lamp, and in the same way the inlet tube *c*. The liquefied agar is then spread upon the walls of the test tube in the usual manner, by turning it upon a block of ice.

I have at times varied this procedure as follows: The liquefied agar jelly, or flesh peptone-gelatine, to which blood or liver tissue, etc., has been added, is first made into an Esmarch roll-tube; the cotton plug (*a*), or a portion of it, is then pushed up into the tube and a rubber stopper (*b*) carrying two short glass tubes is put in position. The end of the test tube is then filled with sealing wax as before. Hydrogen is now passed into the test tube by coupling one of the short glass tubes with a hydrogen apparatus. The test tube is sustained in an inverted position, (see Fig. 3), so that the action of gravity may come into play in displacing the oxygen. This plan is more convenient than the preceding one, and the only objection to it is the fact that a certain amount of oxygen may be retained in the solidified agar jelly covering the walls of the tube. But if the hydrogen is passed for a considerable time it would certainly seem that the greater part of the oxygen must escape by diffusion from this thin layer. Certainly any but the most strictly anaërobic organisms should grow in a tube prepared in this way, if the medium is suitable for it.

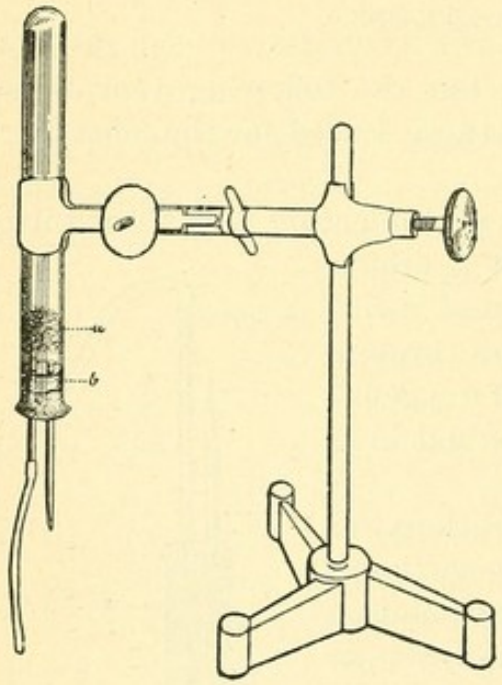


FIG. 3.

I have varied the medium at times by mixing the agar jelly with flesh-peptone-gelatin, or with blood serum. This is easily done when the method last described is employed, but not when the hydrogen is allowed to bubble through the liquified medium; for in this case the addition

of gelatin or of blood serum causes the tube to be filled with bubbles, so that it can not be made into an Esmarch roll-tube.

To add blood serum to our agar or gelatin medium, without contamination which would call for subsequent sterilization, I use the bulbs with a long neck already described as collecting tubes.

Blood from a sheep, or some other suitable animal, is first drawn from a large artery—femoral or carotid—into a sterilized Wolff's bottle, (Fig. 4). The inlet tube *b* is introduced directly into the artery and as the blood flows the air escapes through the cotton filter. When proper precautions are taken the blood in the receiving bottle will be sterile, and after the serum has separated it can be transferred to the bulbs referred to without any danger of contamination, and consequently without any necessity for sterilization by heat. This is accomplished by breaking off the sealed end of the tube *a*, which has previously been notched with a file, and introducing into the clear blood serum the sterilized stem of a collecting tube, the bulb having been gently heated in the flame of a lamp. The serum mounts into the bulb and when it contains a sufficient quantity it is withdrawn and another introduced in its place. Thus one after another is charged, and immediately the extremity of the stem is sealed in the flame of a Bunsen burner or an alcohol lamp. It is a great convenience to have a supply of sterile blood serum always ready, and I keep a stock on hand preserved in this way. Not only are culture liquids preserved in this way safe from contamination but these tubes are convenient to transport, and the transfer of the liquid medium to a test tube is easily and safely effected. Breaking off the end of the stem with sterilized forceps, and introducing it beside the cotton stopper of a sterilized test tube, the contents of the bulb are forced out by applying gentle heat to the portion filled with air.

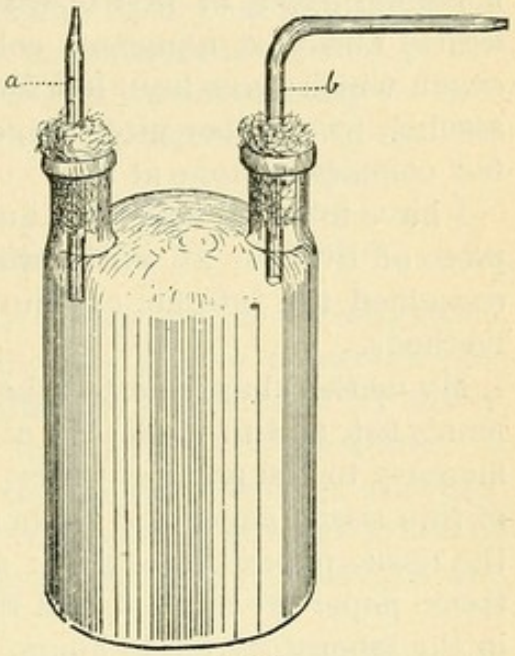


FIG. 4.

I have a small alcohol lamp always at hand for this purpose. In this way I am able to make mixtures of blood serum and agar jelly, or gelatin, and to make Esmarch roll-tubes containing the uncoagulated albumen of the blood serum. This may be a decided advantage under certain circumstances, as our ordinary culture media contain but little albumen after being sterilized at a high temperature.

(*d*) EXAMINATION OF TISSUES KEPT FOR FORTY-EIGHT HOURS IN AN ANTISEPTIC WRAPPING.

In those diseases which have been proved to be due to the presence of microörganisms in the blood or tissues, these microörganisms may

be demonstrated by the methods heretofore referred to and by the examination of properly stained sections of the tissues involved. But in one disease, at least—typhoid fever—cultures from the blood commonly give a negative result, and the bacilli which are present in scattered groups, or colonies, in the spleen, the mesenteric glands, etc., are not always easily found.

Fraenkel and Simmonds* have shown that in the spleen removed from a person dying of this disease the typhoid bacillus multiplies after death, and that numerous colonies may be found in portions of the organ which have been left for 24 to 48 hours before being placed in alcohol, when other pieces placed in alcohol soon after death show but few colonies or none at all.

I have followed this hint, and in most of my autopsies have kept a piece of liver in an antiseptic wrapping for 48 hours, and have then examined the interior of the piece for microörganisms by the usual methods.

My method has been to take a piece of liver or kidney the size of a man's fist, and to wash it in a bath containing 1 part of bichloride of mercury to 500 parts of water. I then envelope it in numerous folds of thin tissue paper and again place it in the antiseptic bath, wetting the tissue paper thoroughly; after which successive wrappings of dry tissue paper are applied, and the piece is placed in a clean jar and kept in the laboratory for 48 hours.

(*e*) EXPERIMENTS UPON ANIMALS.

I have made numerous experiments upon rabbits, guinea-pigs, and dogs, for the purpose of testing the pathogenic power of the various microörganisms which I have obtained from yellow fever cadavers. The results of these experiments will be detailed in the proper place. The method of injecting microörganisms suspended in a liquid into the subcutaneous tissues or abdominal cavity of one of the lower animals which I have found most satisfactory and have practiced for several years, is as follows:

My bulbs with a long stem serve me as a syringe, and I find that they have decided advantages over any form of hypodermic syringe. The piston of the ordinary hypodermic syringe is hard to sterilize, and there is always more or less danger that with this instrument we may inject some other microörganism along with the one which we propose to test. The recent death of a Vienna bacteriologist from the use of a syringe upon himself which he had previously used to inject a culture of the glanders bacillus into one of the lower animals is an unfortunate illustration of this fact. Koch's syringe, which has a rubber ball by which air is forced into the syringe, taking the place of a piston, is much better. But I prefer to employ my improvised syringe, which is still safer, so far as the accidental introduction of microörganisms is

* Die Ætiologische Bedeutung des Typhus-bacillus, 1886.

concerned. This is used but a single time, as I find it easier to make new bulbs than to clean those which have been once used. If the culture has been made in one of these bulbs, it is all ready to inject; if not, it is introduced in the usual way, by gently heating the bulb to form a partial vacuum. To make the injection I first cut away the hair of the animal with scissors, and then with a pair of curved scissors cut away a bit of integument the size of a half-dime or less. The end of the glass stem is drawn out in the flame of a lamp to make a suitable point to the syringe, and this is forced into the subcutaneous tissues, or the cavity of the abdomen. The flame of a small alcohol lamp is now cautiously applied to the bulb, and the liquid is forced out by the expansion of the contained air.

(*f*) EXAMINATION OF TISSUES PRESERVED IN ALCOHOL.

As the liver is the organ which, in the disease under investigation, shows the most constant pathological changes, I have preserved portions of this organ in alcohol for future study at nearly all of my autopsies. I have also preserved in the same way portions of the kidney in a considerable number of cases, and of the spleen, intestine, stomach, and glands from the mesentery in a sufficient number of cases to enable me to study these tissues with reference to pathological changes and the presence of microörganisms.

My thin sections of the tissues have been made with the most approved modern microtomes, and have been stained *secundem artem* by various methods. The staining reagent most extensively employed has been the alkaline solution of methylene blue made according to Loeffler's formula. I have also stained many sections with cabol-fuchsin solution, with aniline-oil-fuchsin (tubercle stain), by Gram's method, by Weigert's method, by the method of Kühne, etc.

(*g*) PHOTOMICROGRAPHS OF MICROÖRGANISMS ENCOUNTERED.

I have made photomicrographs of the microörganisms encountered in my researches, both for the purpose of illustrating my report and as the best method of studying their morphology and comparing one with another.

All bacteriologists now recognize that, as a rule, it is impossible to identify the different species of bacteria by their morphological characters. There are a number of distinct species of micrococci, and of bacilli, which resemble each other so closely in form and dimensions that it is impossible for experts to decide from a microscopical examination alone whether they are identical or not. This can only be determined by other characters, such as growth in various culture media, pathogenic power, etc. But, on the other hand, constant morphological differences enable us to differentiate microörganisms of this class, and such differences are shown in well-made photomicrographs, which enable us to promptly recognize differences of form, of dimensions, and of

arrangement. Measurements are also made with great ease when such photomicrographs have been made with a standard amplification. In those which I have made the amplification has usually been exactly 1,000 diameters. They have been made with the apochromatic objectives and projection eyepieces of Zeiss, and the amplification has been determined by projecting upon the screen the ruled lines upon a stage micrometer made for me by Professor Rogers of Cambridge.

My photomicrographs have mostly been made from fuchsin-stained preparations, and have required the use of orthochromatic plates and a yellow screen.

The screen which I have used is made by adding an alcoholic solution of tropolin to a strong solution of gelatine, boiling to remove the alcohol, and then pouring the gelatine upon a glass plate, where it is allowed to dry. By means of Canada balsam another glass plate is cemented to the one having the stained gelatine coating to prevent this from being scratched.

Some of my photomicrographs have been made by means of the calcium light, but the greater number have been made by gaslight, which has a decided advantage over any other artificial light known to me on the score of convenience and economy. I prefer sunlight and the use of a heliostat, on account of the shorter exposures, but an extended experience has shown me that there are many disappointments when one depends on the light of the sun. On the day we have selected for making our photomicrographs it may be obscured by clouds, and often for weeks together there will be no day suitable for our purpose.

My apparatus for making photomicrographs by gaslight is shown in Fig. 5; *a* is the camera, which has a pyramidal bellows front, sup-

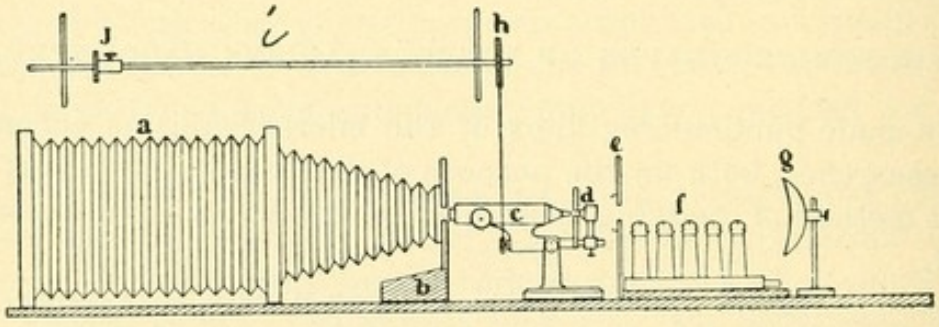


FIG. 5.

ported by the heavy block of wood *b*; this can be pushed back upon the baseboard which supports it, so as to allow the operator to place his eye at the eyepiece of the microscope. When it is brought forward an aperture of proper size admits the outer extremity of the eyepiece and shuts out all light except that coming through the objective; *c* is the microscope, *d* the Abbé condenser supported upon a substage; *e* is a thick asbestus screen for protecting the microscope from the heat given off by the battery of gas-burners *f*. This asbestus screen has an aperture of proper dimensions to admit the light to the condenser *d*.

The gas burners are arranged in a series with the flat portion of the flame facing the aperture in the asbestus screen *e*. The concave metallic mirror *g* is properly placed to reflect the light in the desired direction. I have not found any advantage in the use of a condensing lens other than the Abbé condenser upon the substage of the microscope. With Carbutt's orthochromatic plates, sensitometer 23, and a yellow screen back of the Abbé condenser, I have found that an exposure of from 10 to 15 minutes is required when the screen is in proper position to give an amplification of 1,000 diameters with the apochromatic objective of Zeiss, ol. im., 3mm., ap. 1.30, and his projection eyepiece No. 3.

My arrangement for focusing is also shown in the figure. The brass rod *i* has at one extremity the grooved wheel *h*, which is connected with the fine-adjustment screw of the microscope by means of a cord. The focusing wheel *j* may be slipped along the rod *i* to any desired position, and is retained in place by a set screw. The rod *i* is supported above the camera by arms depending from the ceiling or attached to the baseboard supporting the camera, at such a height that the focusing wheel *j* may be easily reached with the right hand when the operator has his eye at the center of the focusing screen.

IV.—GENERAL RESULTS OF INVESTIGATIONS MADE.

(*a*) EXAMINATION OF "SMEAR-PREPARATIONS."

As stated under the heading "Methods of Research," I have made smear preparations from the material obtained at my autopsies in the entire series of cases. These have for the most part been stained with an aqueous solution of fuchsin.

My preparations of blood from the heart have not shown the presence of microörganisms even in cases in which I have obtained them by the culture method. This is easily explained. When I add two or three drops of blood to liquefied flesh-peptone-gelatine in a test tube and make an Esmarch roll-tube, every microörganism present, capable of growing in this medium, forms a colony. Now, as a rule, the result of such cultures has been negative, but in certain cases colonies of the *bacterium coli commune*, and occasionally of other bacilli, have been developed in these cultures. But the number of colonies has been comparatively small, and the solitary and scattered bacilli which produced them might easily escape attention in a smear preparation, for the amount of blood used in my culture experiments would make very many of these. The same remark applies to similar preparations made from the tissues, but here the result is somewhat different, inasmuch as in a certain proportion of the cases microörganisms have been found in my examination of smear preparations from the liver, made as soon as possible after the autopsy.

This is shown by the following notes, which I transcribe from my note books:

Autopsy No. 14, *Case* 1, 1889.—"Direct examination of material from liver and kidney shows the presence of a large bacillus with square ends." (My bacillus N.)

Autopsy No. 18, *Case* 5, 1889.—"Direct examination of blood negative, of liver a few small, oval bacilli in pairs."

Autopsy No. 22, *Case* 9, 1889.—"Bacilli in liver on direct examination."

Autopsy No. 28, *Case* 15, 1889.—"Material from liver, in collecting bulb, 9 hours after collection, contains a large bacillus. Slide 1325."

Autopsy No. 29, *Case* 16, 1889.—"Numerous bacilli in liver on direct examination (*a*). They are in groups. Slide 1368."

Autopsy No. 33, *Case* 20, 1889.—"Bacillus N present in liver at time of making autopsy. Slide 1426."

Autopsy No. 39, *Case* 26, 1889.—"Bacillus N in liver. Slide 1469."

Autopsy No. 41, *Case* 28, 1889.—"Liver contains a few large bacilli (N). Slide 1476."

As was to have been expected, my smear preparations made from material obtained from the stomach and intestine have always shown

the abundant presence of microörganisms. As in material from the same source obtained from persons dying from other diseases than yellow fever, or from accident, the microörganisms present in the alimentary canal are various and differ to some extent in different individuals. There is no single one to fix the attention as being peculiar to yellow fever, or so constantly and abundantly present as to give ground to the belief that it is concerned in the etiology of this disease.

In the contents of the stomach one finds micrococci and bacilli of various forms and very commonly numerous cells of a torula, resembling in its morphology *torula cerivisæ*.

The micrococci resemble those constantly found in the healthy human mouth. Some are in irregular groups, some in long chains, and some in sarcina-like groups; but no one form is constant. The bacilli also differ greatly in morphology. In one case short oval bacilli in chains were exceedingly abundant; in other cases bacilli resembling the colon bacillus, or larger bacilli in chains, were the most prominent forms present. In the intestine micrococci are not so frequently encountered, and short oval bacilli resembling the *bacterium coli commune* or larger bacilli resembling my bacillus N are more abundant. Evidently no conclusions of any value could be reached by a microscopical examination of these various organisms without resorting to culture experiments, by which they may be isolated and studied separately. This is the difficult task which I felt compelled to undertake and the results of which will be given under another heading. But I may remark here that I have not obtained in my cultures nearly all of the forms which I have recognized in my smear preparations from the stomach and intestine. Some of these I did not obtain at first because I made only aërobic cultures and they prove to be "strict anaërobics." Others have not developed even in my anaërobic cultures, and the inference is that the media employed may not be suitable for their growth. It is quite possible that certain of the bacteria of the intestine depend for their development upon some of the excretory products peculiar to this locality, or upon the presence of other species which evolve products necessary for their growth.

I have had my attention especially attracted by an extremely slender and long bacillus which has been very abundant in many of the smear preparations, but which has never shown itself in my cultures. It is the smallest microörganism, so far as its breadth is concerned, that I have yet encountered; is a flexible filament as shown by the various shapes it assumes, and may reach a length of 50 micromillimetres or more.

(*b*) AËROBIC CULTURES.

My aërobic cultures from blood drawn from one of the cavities of the heart in one of the collecting bulbs described have in a majority of the cases given a negative result; but in a certain proportion of the cases colonies have developed in Esmarch roll-tubes to which one or more drops of blood had been added.

My first five autopsies, made in 1888, gave a negative result. In case 6, autopsy 4 hours after death, colonies of two different kinds were obtained in cultures from the blood, liver, and kidney. One of these was my bacillus *a* (*bacterium coli commune*).

Again, in cases 7 and 8 the result was negative; but in case 9, in which the autopsy was made 5 hours after death, numerous colonies of bacillus *a* developed in my cultures from the blood, liver, and kidney. The next case in which I obtained microörganisms from the blood was No. 15, Havana, 1889. In this case a few colonies of a different bacillus were found.

In case 18, I again obtained a few colonies of bacillus *a*.

Case 19 gave a negative result, and in subsequent autopsies I did not collect blood from the heart, inasmuch as the material obtained from the liver always contained a considerable quantity of blood, and would show the presence of microörganisms if they were to be found in the general circulation.

The results obtained in my aërobic cultures from the liver and kidney are as follows:

In case 1, I obtained a single colony of bacillus *a* in my culture from the kidney. The same bacillus was obtained from the kidney of case 3, and in case 5; the cultures from case 6 gave the same bacillus in gelatine Esmarch tubes from the liver and kidney, associated with another bacillus not particularly described in my notes. In case 9, I obtained numerous colonies of bacillus *a* in cultures from the blood, liver, and kidney. In case 14, a few small transparent colonies developed in my culture from the liver, a few colonies of a micrococcus were obtained from the spleen, and my bacillus *q* was obtained from the spleen and kidney. In case 16 numerous colonies of a bacillus designated by the letter *p*, which I now believe to be identical with my bacillus *x*, were obtained in my gelatine Esmarch tubes from the liver and kidney. This autopsy was a late one, having been made 13½ hours after death. In case 18 a few colonies of bacillus *x* were obtained from the kidney. In case 20 the colon bacillus (*a*) was obtained from the liver; in case 28, my cultures from the liver contained a liquefying bacillus. In case 29 cultures from the liver contained bacillus *a* and bacillus *x*. In case 30 cultures from the liver contained numerous colonies of bacillus *a* and of bacillus *x*. In case 33 numerous colonies of bacillus *a* were obtained in gelatine Esmarch tubes from the liver.

A summary of these results shows that I have obtained microörganisms in my aërobic cultures as follows: In blood from the heart, 4 times in 19 cases; in the liver or kidney or both, 13 times in 43 cases.

It will be noticed that the microörganisms most frequently encountered were non-liquefying bacilli, my bacillus *a* and bacillus *x*.

We are therefore able to assert in the most positive manner that the blood and tissues of yellow fever cadavers do not contain aërobic liquefying organisms, unless by rare exception, and can definitely exclude the

micrococcus of Fréire and the tetragenus of Finlay from consideration as possible agents in the etiology of this disease, as both of these grow readily in the culture medium used in these investigations and both liquefy gelatine.

The non-liquefying bacilli found in a certain proportion of the cases are not in sufficient numbers or sufficiently constant to give support to the view that they are the specific cause of the disease, and the fact that they are not found in a considerable number of typical cases is sufficient reason for excluding them as being directly concerned in its etiology by reason of their presence in the blood and tissues. But we may suppose that they have their proper habitat in the alimentary canal and that the morbid phenomena are due to a toxic ptomaine, produced in this situation. Under this hypothesis the occasional presence of these bacilli in the tissues is to be regarded as accidental and as resulting from the emigration, during the last hours of life or post-mortem, of microörganisms from the intestine, and we have to ascertain whether one of the bacilli found most commonly in the tissues or any other microörganism present in the alimentary canal is the specific agent we are in search of.

AËROBIC CULTURES FROM THE STOMACH AND INTESTINE.

As heretofore stated, direct examination of stained preparations from the stomach and intestine reveals the presence of a vast number and great variety of bacteria. But our aërobic cultures do not enable us to isolate all of the different forms which we may recognize by direct examination. A complete bacteriological analysis of the intestinal contents involves an amount of labor that none but experts in this line of research are likely to comprehend; and as very many of the microorganisms found in this situation will not grow in the presence of oxygen, both aërobic and anaërobic cultures must be made in a variety of media. Moreover, our methods give us only those organisms which are most abundantly present, for these alone remain in the third of a series of plate-cultures, or Esmarch roll-tubes, in which we expect to obtain isolated colonies. In the case of non-liquefying organisms, especially, we have no means of knowing how many species present in No. 1 of a series of Esmarch roll-tubes have been left behind in the diluting process by which we obtain isolated colonies in tube No. 3. The presence of liquefying microörganisms, when these are few in number, is, however, revealed in No. 1 of a series, although the liquefying center may include a variety of microörganisms, and it is only by making a second, and often a third, series of roll-tubes, that we are able to obtain the liquefying organism in pure cultures.

I may say, in general, with reference to my cultures from material obtained from the stomach and intestine post-mortem, and from the alvine discharges during life, that *by far the greater number of the microörganisms present do not liquefy gelatine.* In a series of Esmarch roll-

tubes, No. 1 is sometimes completely liquefied in 24 hours, No. 2 may contain a number of liquefying colonies, but *No. 3 almost without exception contains isolated colonies of non-liquefying bacilli.* In a considerable proportion of the cases there are no liquefying colonies even in tube No. 1 of a series, in which the non-liquefying colonies are so numerous as to give the entire gelatine coating of the tube an opalescent appearance, due to the closely crowded minute colonies. This fact seems to exclude from consideration the supposition that yellow fever is due to the presence in the alimentary canal of a liquefying bacillus, as is the case in cholera.

We might be satisfied with this general statement but for the fact that Dr. Paul Gibier, a French bacteriologist, during his visit to Havana in 1888, encountered a liquefying bacillus which he supposed, for a time at least, to be the specific microbe of the disease. In view of Dr. Gibier's publication referring to this bacillus, I have given special attention to a search for liquefying bacilli in the alimentary canal and in the dejecta. As stated, no liquefying colonies have made their appearance in a considerable proportion of the cases, but in a few exceptional cases the liquefying colonies have been very numerous. Thus in case 2, Havana, 1888, my bacillus *e* was present in my cultures from the stomach. In case 4 and in case 9, Gibier's bacillus (my bacillus *g*) was obtained in my cultures from the stomach and intestine. In case 10 my note is, "single liquefying colony from stomach (Gibier ?)." Case 11, which was an autopsy made 1 hour after death, on the evening of my arrival at Decatur, Ala. (October 3, 1888), gave me very numerous liquefying colonies, both from the stomach and the intestine, and the liquefying organism turned out to be the *Staphylococcus pyogenes aureus.*

In my second autopsy at Decatur (case 12) I again obtained numerous liquefying colonies in my gelatine Esmarch tubes from the stomach and intestine, but this was a very different organism from that encountered in the preceding case; it proved to be a large bacillus with square ends of the "subtilis" order (my bacillus *q*). In a third autopsy, made at Decatur, no liquefying colonies were found in my cultures from the stomach and intestine (case 13). My notes with regard to this point in the autopsies made at Havana in 1889 are as follows:

No. 14. "No liquefying colonies from stomach or intestine." No. 15. "No liquefying colonies in cultures from intestine; a few in Esmarch No. 1 from stomach." No. 16. "Liquefying bacillus (bac. *o*) in gelatine Esmarch tubes from stomach and intestine." No. 17. "Cultures from intestine bacillus *a* and a single liquefying colony, not Gibier's bac." No. 19. "No liquefying colonies from stomach or intestine at end of 24 hours." No. 20. "Bacillus *g*, obtained from intestine. Esmarch's 1 and 2, both liquefied at end of 24 hours." No. 21. "Gelatine Esmarch No. 1 from intestine liquefied at end of 48 hours, bac. *ee*." No remarks in cases 22, 23, and 24. No. 25. "No liquefaction of gelatine Esmarch tubes from intestine." No. 27. "Liquefying bacillus in gel. Es. from intestine." Tube No. 1 liquefied at end of 24 hours." No. 28. "No liquefying colonies in gel. roll-tubes from intestine." No. 29. "Gelatine Esmarch No. 1, from intestine liquefied in 24 hours, single liquefying colony in No. 2, numerous non-liquefying colonies in No. 3, colon, etc." Later note: "The liquefying bacillus from intestine is *g*."

No. 30. "No liquefaction of gelatine Esmarch No. 1 in 48 hours; No. 3 contains numerous colonies of bacillus *x*." No. 31. "No liquefaction of gelatine tubes from intestine at end of 48 hours. No. 3 contains mostly colon bacillus. No. 32. "No liquefaction of gelatine Esmarch No. 1 from intestine in 48 hours." No. 33. Esmarch No. 1, from intestine, contains some liquefying colonies at end of 48 hours (bac. *g*.); none in No. 2 and 3, which contain numerous colonies of the colon bacillus. No. 35. "A few liquefying colonies in Esmarch No. 1 from intestine." No. 36. "No liquefying colonies in Esmarch 1 from intestine." No. 37. "About 100 liquefying colonies in Esmarch No. 1 from intestine; none in 2 and 3, which contain colon bacillus and bacillus X." No. 38. Six liquefying colonies in Es. 1 from intestine; none in 2 and 3.

It will be seen from these notes that Gibier's bacillus (my bacillus *g*) has been present in the intestine in a few of my cases, but that it has been absent in a much greater number, and when present has not been abundant as compared with the non-liquefying organisms. The inference is that its presence is accidental, and that it bears no etiological relation to the disease, and in view of the facts developed by my culture experiments, the broad statement seems justified that *yellow fever is not due to a liquefying aërobic bacillus.*

One reservation might, perhaps, be made before this general statement will be accepted. May not the bacillus of Gibier or some other liquefying bacillus be present in the intestine during the earlier stages of the disease and disappear later, so that it is not found as a constant thing in material obtained from the intestine post-mortem?

Dr. Gibier himself has made the suggestion, and my researches in Decatur were largely made for the purpose of obtaining a definite answer to this question. These researches show that liquefying bacilli were encountered rather more frequently in cultures from the alvine discharges of yellow fever patients than in those from the intestinal contents obtained post-mortem. But the bacillus obtained most frequently was not that of Gibier, but my bacillus *o*. The remarks made with reference to the comparative abundance of liquefying organisms in material obtained from the intestine post-mortem apply as well to the dejecta during life, as compared with the non-liquefying organisms they are very infrequent. This is shown by the following notes made in Decatur:

Feces, case 1: "Esmarch tubes contain in great abundance the colon bacillus, and in much smaller numbers a liquefying bacillus, *o*." Feces, case 2: "Esmarch No. 1 contains a liquefying bacillus, same as in case 1, not very numerous, as shown by absence from tubes 2 and 3." Feces, case 3: "Colon bacillus the principal form; a few liquefying colonies in Esmarch No. 1." Feces, case 4: "Liquefying bacillus in tube 1, and a few colonies in tube 2; bacillus *g*." Feces, case 5: "Liquefying bacillus in tube 1, colon bacillus in tubes 2 and 3 (single liquefying colony in tube 2)." Feces, case 6: "Liquefying colonies in tube 1 (bacillus *g*), colon bacillus in tubes 2 and 3, single liquefying colony in tube 2." Feces, No. 7: "No liquefying colonies; a doubtful case." Feces, case 8: "Numerous liquefying colonies in tube 1; bacillus *o*." Feces, case 9: "Several liquefying colonies in tube 1." Feces, case 10: "No liquefying colonies at end of 48 hours; liquefaction of tube 1 at end of 3 days." Feces, case 11: "Tubes 1 and 2 both liquefied at end of 48 hours." Feces, case 12: "Tubes 1 and 2 both liquefied at end of 48 hours." Feces, case 13: "Tubes 1 and 2 both liquefied at end of 48 hours." Feces, case 14: "No liquefying colonies at end of 48 hours." Feces,

case 15: "Tubes 1 and 2 liquefied at end of 48 hours." Feces, case 16: "Tube 1 partly liquefied at end of 48 hours." Feces, case 17: "Numerous liquefying colonies in tube 1; bacillus *o*." Feces, case 18: "Both tubes liquefied at end of 48 hours." Feces, case 19: "Tube 1 partially liquefied in 48 hours; tube 2 not; bacillus *o*." Feces, case 20: "Tube 1 liquefied in 24 hours; no liquefying colonies in tube 2." Feces, case 21: "Numerous liquefying colonies in 48 hours; bacillus *o* and bacillus *e*." Feces, case 22: "Tube 1 liquefied." Feces, case 23: "Tube 1 liquefied; tube 2 not." Feces, case 24: "Tube 1 liquefied in 24 hours; a few colonies in tube 2; bacillus *o* and bacillus *e*." Feces, case 25: "Both tubes liquefied at end of 24 hours; bacillus *o*." Feces, case 26: "Tube 1 liquefied in 24 hours; tube 2 about 20 colonies; *o* and *t*." Feces, 27: "Tube 1 completely liquefied in 24 hours; No. 2, 20 to 30 colonies." Feces, case 28: "Tube 1 liquefied in 24 hours; no liquefying colonies in tube 2; bacillus *o*." Feces, case 29: "Tube 1 liquefied in 24 hours; single colony in tube 2." Feces, case 30: "No liquefaction in either tube." Feces, case 31: "Tube 1 completely liquefied in 24 hours; bacillus *t*, and another, not *o* or *g*; tube 2 no liquefaction." Feces, case 32: "No liquefaction in 24 hours; tube 1 liquefied in 48 hours." Feces, case 33: "Tube 1 liquefied in 24 hours; all three tubes liquefied in 48 hours; bacillus *g*." Feces, case 34: "Tube 1 not liquefied in 48 hours; some liquefaction on third day; bacillus *o*." Feces, case 35: "No liquefaction in 3 days." Feces, case 36: "A few liquefying colonies in tube 1." Feces, case 37: "Tube 1 contains a few liquefying colonies; bacillus *o*."

It will be noticed that in many instances I have simply noted the fact that liquefaction of tube 1 occurred in 48 hours, etc., without specifying the liquefying organism. This was because I had neither time nor culture material to determine this in every case. The general result of my culture experiments is that liquefying organisms are present in the dejecta in comparatively small numbers, and that there is no one which is constant, but that liquefaction of the gelatine is due sometimes to one and sometimes to another organism of this class.

In a series of comparative experiments made upon the alvine discharges of convalescents and healthy persons liquefying colonies appeared somewhat less frequently, as is shown by the following notes:

October 25: Feces, self. "A single liquefying colony in tube 1." October 25: Feces of convalescents of 3 weeks. "No liquefying colonies." October 26: Feces of healthy man. "No liquefying colonies." October 27: Feces, healthy man. "Neutral reaction; completely liquefied in 3 days." October 27: Feces of convalescent. "About 20 liquefying colonies." October 28: Feces, healthy man. "Completely liquefied on third day." October 28: Feces of convalescent. "Tube 1 completely liquefied in 24 hours; tube 2 on third day." October 28: Feces, healthy man. "Not liquefied." October 28: Feces, healthy man. "Completely liquefied on third day." October 28: Feces, healthy man. "No liquefying colonies." October 29: Feces, convalescent. "Single liquefying colonies." October 29: Feces, convalescent. "Completely liquefied in 48 hours." November 26: Feces, convalescent. "Tube 1 liquefied; bacillus *e*." November 26: Feces, healthy man. "Tube 1 liquefied in 48 hours; a few liquefying colonies in tube 2; bacillus *e*." November 26: Feces, healthy man. "Tube 1 liquefied in 48 hours; some liquefying colonies in tube 2; Wurtzel bacillus." November 26: Feces, healthy man. "Tube 1 liquefied in 48 hours, some liquefying colonies in tubes 2 and 3." November 26: Feces, convalescent. "Tubes 1 and 2 liquefied and a few colonies in tube 3."

It must be remembered that in all these experiments a considerable amount of material is added to tube No. 1, usually 2 or 3 drops from

the collecting bulb, whereas a single *oese* is carried over to tube No. 2, and again from this to No. 3. Evidently, in view of the facts stated, no special significance can be attached to the presence of any one of the liquefying organisms encountered, and the bacillus of Gibier must take its place, besides my bacillus *o*, and my bacillus *e*, as being one of the liquefying organisms occasionally present in the intestinal contents of yellow fever patients.

Inasmuch as nonliquefying bacilli are constantly present in great numbers, our investigation calls for a careful study of this class of organisms, and much of my time has been devoted to this portion of the research.

As already stated, when my cultures from the blood or tissues have given a positive result the microörganisms present have been nonliquefying bacilli, and those most frequently found have been my bacillus *a* and my bacillus *x*. Both of these have been present in my cultures from the intestinal contents, and I can not doubt that this is the source from which they made their way into the blood. I have identified bacillus *a* with the *bacterium coli commune* of Escherich, and it is consequently excluded as the specific cause of yellow fever. Bacillus *x* I have not obtained up to the present time in my comparative researches, and consequently regard it as possibly connected with the etiology of the disease. But I have not been able to obtain any satisfactory experimental evidence upon which to base a positive claim that this is the case. I have not isolated it in a large number of cases, but it was not until my second visit to Havana that I differentiated it from the colon bacillus (*a*) with which it is associated.

In my first culture experiments, made in 1888, I was very much puzzled by the contradictory results which I obtained in inoculating my cultures into rabbits and guinea pigs. I believe now that the promptly fatal results obtained in certain cases in which I injected cultures which I supposed contained only baccillus *a* were due to the presence also of my bacillus *x*. The characters by which these bacilli are to be distinguished, and a detailed account of experiments made with them, will be given in a subsequent portion of this report, in which also a systematic account will be given of some other nonliquefying bacilli encountered in association with them.

In thirteen of my autopsies I have collected urine through the walls of the bladder. My culture experiments with this have given a negative result in ten cases, and in three I have obtained microörganisms, viz: In case 1, "several colonies of bacillus *a* in culture from urine;" in case 2, "the tubes from stomach, intestine, urine, kidney, and spleen all contain numerous colonies of a liquefying bacillus" (my bacillus *e*); in case 9, "numerous colonies of bacillus *a* in cultures from blood, liver, urine, and kidney."

(*c*) ANAËROBIC CULTURES.

My method of making anaërobic cultures has already been described, and an account of the various microörganisms which I have isolated by

this method will be given hereafter. The general result has been, so far as the blood and tissues are concerned, similar to that obtained in my aërobic cultures. That is, various microörganisms have been encountered in the series of cases in which this method has been applied, but no one of them has been constant, and in a considerable proportion of the cases the result has been entirely negative.

Some of the microörganisms isolated in my anaërobic cultures are identical with those obtained in aërobic cultures from the same source; for my bacillus *a*, bacillus *x*, and other bacilli associated with them in the intestine are facultative anaërobics, and grow either in the presence or in the absence of oxygen. But there are also in the intestine, and in the tissues obtained post mortem at a considerable interval after death, a number of "strict anaërobic" bacilli. Some of these I have isolated and shall describe hereafter.

The following extracts from my note book will show the general results obtained by this method.

Case 14, *Havana, April* 23, 1889: Soldier in military hospital. Sick 5 days; autopsy 9 hours after death. "Direct examination of material from liver and kidney shows a large bacillus with square ends (bacillus N). Numerous granular colonies in anaërobic culture from liver (N). Five liquefying colonies in anaërobic culture from kidney."

Associated with my bacillus N in the anaërobic cultures from the liver of this case was a microörganism in chains, which I was at first disposed to regard as a short bacillus and designated with the letter *o*. This I isolated and have still in cultivation. I now regard it as a streptococcus.

Case 15, *April* 28, 1889: Sick 10 days; autopsy 9 hours after death. "No microörganisms on direct examination of material from liver and kidney. No development in aërobic or anaërobic Esmarch tubes from liver and kidney." *Case* 16: Sick 7 days; autopsy 13½ hours after death. Numerous colonies of bacillus *x* in gelatine cultures from liver and kidney; anaërobic cultures from liver and kidney contain the same bacillus. *Case* 17: Sick 5 days; autopsy 5 hours after death. No colonies either in aërobic or anaërobic cultures from blood, liver, kidney or urine." *Case* 18: Sick 5 days; autopsy 2 hours after death. "Direct examination of blood negative; of liver a few small oval bacilli in pairs. Aërobic gelatine Esmarch tube from blood contains a few colonies of bacillus *a*. Anaërobic agar Esmarch tube from blood contains numerous colonies of bacillus *a* and of a short bacillus in chains, slide 1078. Anaërobic agar Esmarch tube from kidney contains numerous colonies; bacillus *w*, the same from liver." *Case* 19: "No colonies in gelatine Esmarch tubes from blood or liver; no development in gelatine anaërobic tube from blood." *Case* 21: Sick seven days; autopsy 10 hours after death. "No colonies in gelatine Esmarch tube from liver; numerous colonies of bacillus N in anaërobic agar Esmarch tube." *Case* 24: Sick 5 days; autopsy 4½ hours after death. "No development in anaërobic cultures from liver and kidney." *Case* 30: "Aërobic cultures from liver contain both bacillus *a* and bacillus *x*. Anaërobic culture from liver contains bacillus N and bacillus O." *Case* 31: "No development in aërobic or anaërobic cultures from liver." *Case* 32: "No development in aërobic culture from liver, bacillus N in anaërobic cultures from liver." *Case* 35: "No microörganisms in liver on direct examination; no development in aërobic or anaërobic cultures from liver." *Case* 36: "The anaërobic culture from the liver in this case gives numerous colonies of a facultative anaërobic bacillus." *Case* 37: No colonies in anaërobic culture from liver. *Case* 38: "A few colonies of bacillus *x* in anaërobic culture from liver."

(*d*) RESULTS OF EXAMINATION OF TISSUES KEPT IN AN ANTISEPTIC WRAPPING.

Upon removing the antiseptic wrapping from a piece of liver or kidney preserved as heretofore described, I have usually found the exterior of the piece to be thoroughly sterilized and free from any signs of putrefactive change. The interior, also, usually has a fresh appearance and is without any putrefactive odor. But a microscopical examination of a stained smear preparation shows that a large number of microorganisms are present, although smear preparations from the same material made immediately after death have as a rule given a negative result. There is evidence then that the liver and the kidney of yellow fever patients does contain microörganisms when removed from the cadaver at an autopsy made within a few hours after death. For the numerous microörganisms of various species found in a piece preserved in this way have evidently developed from others which were present in small numbers at the time it was removed from the body. The method insures the destruction of any bacteria which might accidentally fall upon the fragment after opening the cavity of the abdomen; and, as stated, the exterior of the fragment is perfectly preserved by the antiseptic wrapping. Comparative experiments made with pieces of liver obtained from persons dying with other diseases have given a similar result, and, even in a case of sudden death, in which the autopsy was made almost immediately, I have found numerous microörganisms.

The one which I found most constantly and most abundantly in yellow-fever tissues preserved in this way was a large anaërobic bacillus—my bacillus N, which I now call *bacillus cadaverinus*. Having also found this several times in my smear preparations from fresh liver tissue, and finding it to be very common in the contents of the intestine, I hoped for a time that it might turn out to be the specific infectious agent in the disease under investigation. But before leaving Havana, I had already found what appeared to be the same bacillus in a piece of liver, preserved in the same way, which I obtained from a case of tuberculosis; and since my return to Baltimore I have found it in other comparative autopsies; so that I now feel compelled to exclude it from consideration as having any etiological relation to yellow fever.

A number of the bacilli which I have obtained from yellow fever cadavers, and shall hereafter describe, have been from pieces of liver and kidney preserved in the manner mentioned; and have been isolated either directly by means of Esmarch roll-tubes, or indirectly by inoculations into guinea-pigs.

These bacilli are either strict or facultative anaërobics, and they are all able to grow in a decidedly acid medium. Several of them produce an acid reaction in glycerine agar tubes, and one (bacillus *i*) produces a very acid reaction in bouillon cultures to which 5 per cent. of glyce-

rine has been added. I therefore ascribe the very acid reaction of the interior of the piece of tissue, kept as indicated, to the presence of these acid-producing bacilli. I give below some extracts from my notes relating to this material kept in an antiseptic wrapping for 48 hours:

Case 1, *Havana*, *May* 12, 1888: "May 14, examined liver and kidney kept in laboratory for 48 hours. On section there is no putrefactive odor. The kidney contains a large bacillus with square ends—slide 230; the liver contains the same bacillus—slide 231." *Case* 18, *May* 13, 1889: "Liver kept in laboratory 48 hours has on cut surface an intensely acid reaction; kidney also has intensely acid reaction; both contain bacillus N and a small oval bacillus (slide 1081)." *Case* 23, *June* 4, 1889: "Liver 48 hours in laboratory; no odor, slightly acid reaction, bacillus N not present." *Case* 28: "Liver 36 hours in laboratory contains bacillus N in large numbers—slide 1336; has no odor and an acid reaction." *Case* 29: "Liver 48 hours in laboratory has an acid reaction, no odor, and contains bacilli resembling *x*—slide 1375. Both colon bacillus and bacillus *x* obtained from this liver kept 48 hours in laboratory, also bacillus of rabbit septicæmia by inoculations." *Case* 30: "Liver 48 hours in laboratory contains bacillus N and other bacilli—slide 1414; acid reaction." *Case* 31: "Liver 48 hours; acid reaction, bacillus N—slide 1417." *Case* 33: "Liver 48 hours; bacillus N, acid reaction, fresh appearance." *Case* 34: "Liver 48 hours; contains bacillus N in small numbers, fresh appearance, no odor, acid—slide 1437." *Case* 35: "Liver 48 hours; bacillus N—slide 1442; very acid reaction, no odor, fresh appearance." *Case* 36: "Liver 48 hours, acid, fresh appearance, no odor, bacillus N—slide 1455." *Case* 37: "Liver 48 hours in laboratory; putrefaction has commenced; various bacilli present; reaction faintly acid." *Case* 38: "Liver 48 hours; fresh appearance, no odor, acid reaction, contains bacillus N—slide 1468." *Case* 39: "Liver 48 hours, bacillus N." *Case* 40: "Liver 48 hours; putrefaction commencing; various bacilli including N—slide 1477."

COMPARATIVE AUTOPSIES.

No. 1, *Havana*, *May* 17, 1889: "Liver and kidney from case of tuberculosis; autopsy 2 hours after death. May 19, cut surface of liver has a very acid reaction and contains bacillus N in large numbers; kidney contains some bacillus in smaller numbers; bacillus *x* not present."

No. 2, *Havana*, *May* 19: "Autopsy 1¾ hours after death; tuberculosis; kidney at end of 48 hours fresh; no microörganisms; liver, putrefaction; cut surface, acid; contains a large bacillus with round ends; bacillus N not present—slide 1120."

No. 3, *Havana*, *May* 22: Case of heart disease. "Liver at the end of 48 hours contains bacillus N; cut surface has a very acid reaction; bacillus *x* not present."

No. 4, *Havana*, *May* 25: Abscess of liver. "Autopsy one-half hour after death. No microörganisms on direct examination at end of 48 hours; staphylcoccus obtained in cultures; cut surface of liver has a slightly acid reaction."

No. 5, *Havana*, *May* 25: Insane woman. "Autopsy 5 hours after death; putrefactive decomposition of liver kept 48 hours in antiseptic wrapping."

No. 6, *Havana*, *May* 30: Tuberculosis. "Autopsy 6 hours after death; bacillus N not present; slightly acid reaction."

No. 7, *Havana*, *June* 2: Heart disease; autopsy 5 hours after death. "Liver putrid at end of 48 hours."

No. 8, *Baltimore*, *October* 30: Tuberculosis; autopsy 8 hours after death. "Liver preserved 48 hours in antiseptic wrapping has a fresh appearance, no odor, acid reaction; contains large bacillus with round ends and end spore—slide 1485."

Case 9, *Baltimore*, *November* 12, 1889: Tubercular meningitis; autopsy 6½ hours after death. "Liver kept for 48 hours in laboratory has an acid reaction and contains various bacilli; one very large with round ends, one long and slender—slide 1505; bacillus N not present."

Case 10, *November* 18, 1889: Osteomyelitis of tibia, amyloid liver, and kidney. "Liver 48 hours in laboratory is perfectly preserved, has a fresh appearance and no odor, very acid reaction. Contains numerous and various bacilli, one resembling N."

No. 11: Tuberculosis; autopsy 24 hours after death. "Liver 48 hours in laboratory very soft, and has an empyreumatic odor; contains a large bacillus with round ends; bacillus N not present."

Case 12: Tuberculosis; autopsy 8 hours after death. Liver in oven at 35° for 48 hours; soft, putrefactive odor; alkaline reaction; various bacilli present, N not recognized.

Case 13: Death from chloroform; autopsy at once. "Liver preserved in antiseptic wrapping; opened at end of 48 hours has an acid reaction and fresh appearance; contains a large anaërobic bacillus with end spores—slide 1543."

Case 14: Heart disease; autopsy 7 hours after death. "Liver, 48 hours, fresh appearance, no odor, acid reaction; contains various bacilli—slide 1550."

Case 15: Peritonitis after laparotomy. "Liver in antiseptic wrapping at end of 48 hours is perfectly fresh in appearance, has a very acid reaction, and contains large anaërobic bacilli in great numbers which appear to be identical with my bacillus N. These were not obtained in an anaërobic culture in glycerine agar, but were present in an anaërobic culture in blood serum and glycerine agar mixed, associated with other bacilli—slide 1898."

Case 16: Tumor of uterus; autopsy 4 hours after death. "Liver preserved 48 hours, fresh in appearance, no odor, very acid reaction; various bacilli; bacillus N not present."

These notes show that the large anaërobic bacillus which I have designated by the letter N was more constantly present in the liver from my yellow-fever cases than in my comparative autopsies, but the hope which I entertained for a time that it might be the specific infectious agent has given way before the evidence of its presence in the liver of persons dying from other diseases. It is true that I had this evidence before returning from Havana, but I admitted to myself the possibility that acclimated persons dying in the infected city during the epidemic season might carry the specific germ in their intestine, and that upon their death from another disease it might invade the tissues. I was therefore not willing to exclude this bacillus until I had found it in comparative autopsies made outside of the area of possible yellow-fever infection. Having found it in my comparative autopsy No. 15, made in the city of Baltimore, in January, 1890, I can no longer entertain the supposition that it may be concerned in the etiology of yellow fever.

(*e*) RESULTS OF EXPERIMENTS UPON ANIMALS.

I shall give an account of my experiments with pure cultures of the various bacteria which I have isolated in connection with my systematic description of these microörganisms. At present it is my intention to record the results obtained from inoculations of blood, urine, liver tissue, and material from the stomach and intestine, and first it will be well to call attention to similar experiments which have been made by others. I quote from my previous report, published in the annual volume of the Marine-Hospital Service for 1889.

We quote as follows from Dr. Freire's principal work (Doctrine Microbienne de la Fievre, Jaune, 1885):

"My first experiments made upon the monkey and the dog gave a negative result (p. 35).

"I have also inoculated black vomit into a dog, repeating the injection twice, at intervals of some days. * * * No phenomenon indicative of yellow fever manifested itself (p. 36).

"Fowls and pigeons also enjoy a complete immunity, as we shall see further on. After having inoculated these animals with blood drawn directly from the corpses of yellow-fever patients, and also with cultures of different degrees of transplantation, without having succeeded in any case in transmitting to them the malady, I turned my attention to other animals, to rabbits and to guinea-pigs.

"My attention was especially called to guinea-pigs because a merchant of the place said to me that just when the epidemic had attained its maxium of intensity he supported an enormous loss on account of the *peste*, which killed each day a great number of his guinea-pigs" (p. 36).

We remark that the guinea-pig is very subject to various forms or septicæmia, and that those who have endeavored to raise them in latitudes where yellow fever does not prevail have often experienced heavy losses, especially during hot weather and when their cages are not carefully cleaned. My guinea-pigs in Havana in 1879 did not contract yellow fever, although they were exposed on an infected ship during the hottest part of the year for a period of 48 hours. Moreover, Dr. Freire himself gives evidence that during the winter months he inoculated these little animals with blood from yellow fever patients without result. He says:

"The influence of season upon the evolution of the microbe of yellow fever is very powerful. For the purpose of determining the nature of this influence we have proceeded to various experimental researches. We have inoculated a large number of guinea-pigs, not only by the method of vaccination but also by subcutaneous injection of cultures of the microbe in gelatine. These cultures showed themselves fertile in characteristic organisms, and their energy had already been proved, since their inoculations had caused the death of several animals. Very well; these inoculations made in July and August have given only negative results. The animals presented a slight elevation of temperature, but survived the consequences of the inoculation. Even the blood of patients sick with yellow fever inoculated into animals in the months of July and August could not cause their death. Indeed, on the 15th of August we have injected with the blood of a patient attacked with yellow fever nine guinea pigs. The following is the course of the temperature as observed:

Number.	Before the experiment.	After injection.			
		July 16.	July 17.	July 18.	July 19.
	°	°	°	°	°
1	38.9	39.0	37.8	38.2	39.0
2	38.8	38.7	39.0	39.0	39.6
3	39.0	39.0	38.0	38.4	38.8
4	38.6	39.9	38.9	38.8	38.6
5	39.0	39.2	38.0	39.9	39.0
6	38.8	39.0	38.8	39.8	40.0
7	39.1	39.0	38.5	38.9	39.4
8	39.2	39.2	37.4	39.0	38.5
9	38.8	38.2	39.6	39.2	38.5

"The following days the temperatures of nearly all of these animals became normal. None of them died. The fact shows the innocuity, due to a change of season, of the inoculations which a month previously showed themselves virulent and so toxic that they infallibly caused the death of all the animals" (p. 235).

Dr. Freire has referred to the experiments of Dr. Rangé, "Médecin de première classe," of the French navy, as confirming his own. These experiments were made during an epidemic, which occurred in 1885, upon the Iles du Salut (Guayana).

The inoculation experiments of Dr. Rangé were made *during the height of the epidemic*, in the month of April. He says:

"In guinea-pigs inoculation of blood taken directly from the patient was not followed by any result. Inoculations with *black vomit, cultures from blood, or cultures from black vomit* gave always a positive result; that is to say, they were followed by reactional phenomena. Four times they determined death."

These cultures, like those of Dr. Freire and others obtained from the same source, no doubt contained various organisms, and among them one or more may have been pathogenic for the guinea-pig; but the experiments made have shown most definitely that blood drawn directly from the patient during the epidemic season does not kill guinea-pigs. We must therefore conclude that the death of guinea pigs inoculated by Dr. Freire during the epidemic season resulted not from yellow fever, but from inoculation with some pathogenic organism which was abundant during the summer months, and consequently was present in his cultures, or from accidental inoculation through the wound made by him in his experiments. The guinea-pig is very subject to the last-mentioned accident, especially when kept in foul cages. Its own discharges and the remnants of food in its cage furnish a pabulum in which a multitude of microörganisms are to be found. Owing to the shortness of its legs its abdomen is constantly soiled with this material, and if any pathogenic organism is present an inoculation wound made for experimental purposes can scarcely fail to be infected with it.

It is scarcely worth while to give in detail the experiments upon guinea-pigs made by Freire in which death followed the inoculation, and in every one of which the assumption is made that the animals succumbed to yellow fever. But his summary statement of these experiments presents some points of interest. Thus we find that one animal died at the end of a few hours, while one lived for 30 days. Yet death in both of these extreme cases is ascribed to yellow fever, resulting from the inoculation practiced.

BLOOD AND LIVER TISSUE FROM A RECENT AUTOPSY NOT PATHOGENIC FOR GUINEA-PIGS OR RABBITS.

My experiments made in Havana *during the epidemic season* fully confirm those of Rangé as to the innocuity of yellow fever blood when injected into guinea-pigs in considerable quantity. This is shown by the following experiments:

May 13, 1889.—Injected one-fourth of a cubic centimetre of blood obtained at autopsy from heart of case 7 into the abdominal cavity of a very small guinea-pig. Result negative.

May 13, 1889.—Injected one-half of a cubic centimetre of blood serum from heart of case 17 into cavity of the abdomen of another small guinea-pig. Result negative.

May 23, 1889.—Injected subcutaneously and also in cavity of abdomen a small amount of crushed liver tissue from case 19 into guinea-pig No. 54. No result.

May 26, 1889.—Injected into subcutaneous tissue of guinea-pig No. 58 one-half of a cubic centimetre crushed liver tissue from case 20. No result.

June 4, 1889.—Injected subcutaneously into guinea-pig No. 82 5 minims of crushed liver tissue from case 22. No result.

June 13, 1889.—Injected subcutaneously into guinea-pig No. 100 one-half of a cubic centimetre blood and crushed parenchyma from liver of case 24. No result.

June 13, 1889.—Injected subcutaneously into guinea-pig No. 101 one cubic centimetre blood and crushed tissue from kidney, case 24. No result.

June 29, 1889.—Injected subcutaneously into guinea-pig No. 126 one-half of a cubic centimetre blood and liver pulp from case 25. No result.

All of these injections were made with material in which no micro-organisms were recognized in smear preparation stained with fuchsin. In the following cases in which death followed the inoculation micro-organisms were present:

May 27, 1889.—Injected subcutaneously into guinea-pig 60 (quite small) 4 minims of material from liver of case 21, just collected. This animal was found dead at 6 a. m., on the morning of May 28. The autopsy showed extensive subcutaneous œdema extending from point of inoculation, and the effused serum contained a large anaërobic bacillus, my bacillus N.

July 16, 7.30 *a. m.*—Injected subcutaneously into guinea-pig No. 153, 5 minims of blood from liver, case 28, containing a large bacillus; slide 1325 (N ?). The animal died at 10 p. m. Extensive subcutaneous effusion of bloody serum. Bacillus N recovered from liver.

These experiments show that blood and liver tissue obtained at recent autopsies do not, as a rule, kill guinea-pigs, but that in exceptional cases, in which the large anaërobic bacillus is present, which I have designated by the letter N, death may occur very promptly.

I have also obtained negative results from injections of fresh liver tissue into rabbits.

August 9, 1889.—Injected subcutaneously into rabbit No. 158 2 minims of crushed liver tissue from case 30. Result negative.

August 12, 1889.—Injected subcutaneously into rabbit 164 5 minims of material from liver of case 32. Result negative.

August 13, 1889.—Injected subcutaneously into rabbit 189 4 minims of material from liver of case 33. Contains bacillus N, slide 1426. Result negative.

August 15, 1889.—Injected subcutaneously 1 cubic centimetre material from liver of case 35, principally blood, into rabbit 170. Result negative.

August 19, 1889.—Injected subcutaneously one-half of a cubic centimetre material from liver of case 36 into rabbit 178. Result negative.

August 21, 1889.—Injected subcutaneously into rabbit 183 2 minims material from liver of case 37. Result negative.

August 22, 1889.—Injected subcutaneously 1 cubic centimetre blood and liver tissue from case 38 into rabbit 184. Result negative.

These experiments suffice to show that as a rule blood and liver tissue from a recent autopsy is not pathogenic for rabbits. But in the

following case death resulted from the subcutaneous injection of similar material:

August 10, 1889.—Injected subcutaneously into rabbit 159 3 minims from liver of case 31. Animal died in convulsions at 1 p. m., August 12.

The bacillus of rabbit septicæmia was recovered from the liver in an agar-stick culture.

The same bacillus was obtained indirectly from another case, as follows:

Guinea-pig No. 172 injected July 31 with material from liver of case 29, kept 48 hours in an antiseptic wrapping, died the following day. Anaërobic culture in glycerin agar from the liver of this guinea-pig contained bacilli and was injected (1 cubic centimetre) into guinea-pig 144, which died at the end of 32 hours from the time of injection. An anaërobic culture in blood serum from the liver of this guinea-pig was injected on August 6 into rabbit 150, which died the following day. The blood and liver of this rabbit contained a small bacillus with stained ends, which proved to be the bacillus of rabbit septicæmia. Cultures from the blood and liver of this rabbit and the preceding one were subsequently injected into other rabbits with a uniformly fatal result, and the bacillus was fully identified as Koch's bacillus of rabbit septicæmia, now generally admitted to be identical with the bacillus of fowl cholera, first described by Pasteur as a micrococcus.

YELLOW FEVER URINE NOT PATHOGENIC FOR RABBITS.

May 5, 1889.—Injected into cavity of abdomen of rabbit 101 7 cubic centimetres urine from case 16, drawn at autopsy through walls of bladder. Result negative.

May 10, 1889.—Injected into cavity of abdomen of rabbit 105 (weight 1,000 grammes) 11 cubic centimetres of albuminous yellow fever urine from a case in the third day of the disease. Temperature, 30 minutes after the injection, 104° F.; respiration normal. The animal remained in good health until used for another experiment.

July 29, 1889.—Injected into cavity of abdomen of rabbit 133 (weight about 1,000 grammes) 10 cubic centimetres of highly albuminous urine from a case in the fourth day of the disease; urine collected two hours before injection; slightly acid; specific gravity, 1.025. Result negative.

VIRULENCE OF LIVER TISSUE KEPT FOR 48 HOURS IN AN ANTISEPTIC WRAPPING.

I have already referred to the fact that a piece of liver or kidney from a yellow-fever patient which has been enveloped in an antiseptic wrapping at the time of the autopsy contains numerous microörganisms when the antiseptic wrapping is removed at the end of 48 hours. Now, if a little material from the interior of one of these pieces is injected beneath the skin of a guinea-pig my experiments show that death generally occurs within a comparatively short time. Very shortly after the injection is made the animal becomes restless, and at the end of 3 or 4 hours he commences to dash about his cage at intervals in an abrupt, "nervous" way. Death frequently occurs inside of 24 hours,

and at the autopsy an extensive accumulation of bloody serum is found in the subcutaneous connective tissue. This gravitates to the most dependent position, and commonly the entire wall of the abdomen is filled with the bloody fluid, which accumulates to such an extent as to form a large fluctuating pouch.

The effused bloody serum usually contains various bacilli, among which the most conspicuous is my bacillus N, which is sometimes present in great numbers and almost in a pure culture. This bacillus does not invade the blood and is not found in smear preparations or cultures from blood taken from the heart or material from the liver. Cultures from the liver or blood, however, almost always give one or more bacilli, which have been associated with bacillus N in the liver tissue, preserved for 48 hours, and which was used for the inoculation. Very commonly I have obtained in these cultures my bacillus *a* or bacillus *x*, or both of these.

Two or three minims of the bloody serum present in the subcutaneous tissue injected into another guinea-pig also causes death with the same symptoms and pathological appearances, and in the same way a third may be inoculated from the second, and so on. But the virulence appears to diminish, the time before death being prolonged in the case of the second and third animals in a series, and beyond this a fatal result is not always produced.

NOTES OF EXPERIMENTS MADE IN HAVANA IN 1889.

May 14, 4 *p. m.*—Injected subcutaneously into guinea-pig No. 43 a small amount of crushed liver parenchyma from piece kept 48 hours in antiseptic wrapping, from case 18. The animal was found dead at 6 o'clock a. m., May 16. There was an extensive collection of bloody serum in the subcutaneous connective tissue, which contained bacillus N in large numbers and other bacilli. The liver was dark in color and rather soft, spleen normal.

May 16, 10 *a. m.*—Injected subcutaneously 3 minims of bloody serum from subcutaneous connective tissue of guinea-pig No. 43, into guinea-pig No. 45. Died morning of May 21, extensive subcutaneous œdema; liver and spleen normal and do not contain bacilli.

May 24, 4 *p. m.*—Injected subcutaneously into guinea-pig No. 56, 2 minims from liver of case 19, kept 48 hours in laboratory. Found dead at 6 a. m., May 29. No subcutaneous œdema; gall bladder much distended; no microörganisms in liver; abdominal viscera normal in appearance. Pure culture of bacillus *x* obtained from blood of heart.

July 5, 1 *p. m.*—Injected subcutaneously into guinea-pig 100, 2 minims of material from liver of case 27, 48 hours in antiseptic wrapping. July 6, 4 p. m. the animal dying and was killed. Slight subcutaneous œdema. Abdominal viscera normal in appearance. Pure culture of bacillus *x* obtained from blood of heart.

July 31, 9:30 *a. m.*—Injected subcutaneously into guinea-pig No. 172, 2 minims of material from liver, case 29, 48 hours in antiseptic wrapping. Died at 11:30 a. m. the next day. No subcutaneous œdema; spleen very large and dark in color; small intestine hyperæmic and contains a bloody fluid. No microörganisms in culture from liver and blood from heart in aqua coco. Anaërobic culture in aqua coco contains a bacillus having vibrio-like movements.

August 11, 10 *a. m.*—Injected subcutaneously into guinea-pig 185, 3 minims from

liver of case 30, 48 hours in antiseptic wrapping. Dead August 13, at 6 a. m. Extensive subcutaneous œdema. Cultures in aërobic gelatine tubes from subcutaneous bloody serum contain colon bacillus.

August 15, 2:30 *p. m.*—Injected subcutaneously into guinea-pig No. 190, 4 minims of material from liver of case 21, 48 hours in antiseptic wrapping; contains bacillus N. Animal found dead at 6 a. m. the next morning.

August 21, 11:30 *a. m.*—Injected subcutaneously 1 minim from liver of case 36, preserved for 48 hours in antiseptic wrapping. No result.

August 24, 12 *m.*—Injected subcutaneously into guinea-pig 187, 2 minims of material from liver of case 38, preserved 48 hours in an antiseptic wrapping. Found dead at 6 a. m. the next morning. Usual subcutaneous œdema containing bacillus N slide 1470.

EXPERIMENTS UPON RABBITS.

August 11, 1889.—Injected subcutaneously into rabbit, No. 161, 4 minims of material from liver of case 17, kept 36 hours in antiseptic wrapping; contains bacillus N. Result negative.

NOTE:—Three minims of the same material killed guinea-pig No. 185.

August 17, 1889.—Injected subcutaneously 4 minims of material from liver of case 35, kept for 48 hours in an antiseptic wrapping, into rabbit No. 176. No subcutaneous œdema. A motile bacillus recovered in aërobic culture from liver; slide 1461.

July 30, 3:30 *p. m.*—Injected subcutaneously into rabbit 136, 10, minims of bloody serum from subcutaneous connective tissues of guinea-pig 134; contains bacillus N and other bacilli; slide 1369. Animal found dead at 5 a. m. the next day. Some subcutaneous œdema.

July 31, 9 *a. m.*—Injected subcutaneously into rabbit 139, 2 minims of bloody serum from cellular tissue of rabbit 136. Animal died at 9 p. m. the same day. Very little subcutaneous œdema.

August 11, 10 *a. m.*—Injected subcutaneously into rabbit No. 161, 4 minims of material from liver of case 17, preserved 36 hours in an antiseptic wrapping; contains bacillus N. Result negative.

August 11, 3 *p. m.*—Injected subcutaneously into rabbit 162, 5 minims bloody serum from cellular tissue of guinea-pig 150, just dead, containing bacillus N and other bacilli; slide 1415. The animal died at 10:30, August 15; abscess in middle of belly, liver dark in color, spleen normal. The liver of this rabbit, kept 48 hours in antiseptic wrapping; had on acid reaction and contained bacillus N.

August 17, 9:30 *a. m.*—Injected subcutaneously into rabbit No. 176, 4 minims of material from liver of case 22, 48 hours in antiseptic wrapping; contains bacillus N; slide 1442. Dead August 21 at 6 a. m. A motile aërobic bacillus obtained in culture from liver; slide 1461. No subcutaneous œdema.

COMPARATIVE EXPERIMENTS.

June 1, 1889, 10 *a. m.*—Injected subcutaneously into guinea-pig No. 72, 3 minims of material from liver of case of tuberculosis, kept 48 hours in antiseptic wrapping; slide 1187 (comparative autopsy No. 6). Animal found dead the next day at 6 a. m. Extensive collection of bloody serum containing bacilli in subcutaneous tissues; slide 1193.

November 20, 1889, 3 *p. m.*—Injected subcutaneously into guinea-pig No. 173, a little material from interior of piece of liver kept in antiseptic wrapping for 48 hours, from comparative autopsy No. 10; slide 1517. Animal found dead at 9 a. m. the next day. Extensive subcutaneous œdema; containing various bacilli; slide 1581. Spleen enlarged; no microörganisms recognized in smear preparation.

November 22, 10:30 *a. m.*—Injected sub-cutaneously 2 minims bloody serum from connective tissue of guinea-pig No. 173 (above) into guinea-pig No. 174. Animal died

at 3:30 p. m., November 23. Considerable subcutaneous œdema (slide 1526), from which cultures in gelatine roll tubes give a liquefying bacillus of the proteus group.

November 30.—A culture of this liquefying bacillus in agua coco, injected subcutaneously into guinea pig No. 176, gave a negative result.

November 23, 4 *p. m.*—Injected subcutaneously 3 minims bloody serum from cellular tissues of guinea-pig No. 174. Animal found dead on the morning of November 25. Extensive subcutaneous œdema containing bacilli (slide 1527). Liquefying bacillus recovered from liver.

November 25.—Injected subcutaneously 2 minims bloody serum from guinea-pig No. 177. Animal recovered.

November 30, 5 *p. m.*, 1889.—Injected subcutaneously 2 minims material from liver of comparative autopsy No. 13, preserved 48 hours in antiseptic wrapping, into guinea pig No. 181. Dead next morning at 9 o'clock. Extensive subcutaneous effusion of bloody serum with separation of integument; bloody serum contains a long bacillus (slide 1544).

December 2. 3:30 *p. m.*—Injected subcutaneously 3 minims bloody serum from cellular tissue of guinea-pig No. 181, into guinea-pig No. 182 (material had been kept 24 hours in laboratory in the sterilized collecting bulb). Animal found dead at 9 a. m., December 5. Extensive subcutaneous collection of bloody serum which contains various bacilli (slide 1556). Gelatine roll-tubes contain a liquefying bacillus, a proteus.

December 5, 11 *a. m.*—Injected subcutaneously 3 minims of bloody serum from cellular tissue of guinea-pig 182, into guinea-pig No. 185. Found dead next morning. Some subcutaneous œdema (slide 1559).

December 6, 9 *a. m.*—Injected subcutaneously into guinea-pig 186, 3 minims of bloody serum from cellular tissue of guinea-pig 185. Animal recovered.

January 4, 3 *p. m.*, 1890.—Injected subcutaneously 3 minims of material from liver, comparative autopsy No. 14, kept 48 hours in antiseptic wrapping, into guinea-pig 191; contains a large bacillus resembling N; slide 1592. Animal died next day at 3 p. m. Extensive collection of bloody serum in subcutaneous connective tissue containing bacillus N; colon bacillus obtained in culture from liver.

January 5, 5 *p. m.*—Injected subcutaneously into guinea-pig 193, 4 minims of bloody serum from cellular tissue of guinea-pig 191. Animal very feeble and killed at 5 p. m., January 6. Extensive subcutaneous œdema; bloody serum contains bacillus N.

January 6,5 *p. m.*—Injected subcutaneously into guinea-pig 194, 4 minims of bloody serum from cellular tissue of guinea-pig 193. Found dead at 9 a. m., January 8. No subcutaneous œdema; liver dark in color and full of blood; spleen enlarged; colon bacillus recovered from liver.

These comparative experiments suffice to show that the *virulence of liver tissue kept for* 48 *hours in an antiseptic wrapping is not peculiar to yellow fever.*

MATERIAL OBTAINED FROM THE STOMACH SOON AFTER DEATH IS VIRULENT FOR GUINEA PIGS.

The stomach is usually found to contain a considerable quantity of fluid, which may correspond with that ejected during the last hours of life—"black vomit"—or may be a grumous and thick fluid, having a brownish color, and containing a variety of microörganisms, together with the desquamated epithelium of the stomach.

Aërobic cultures from this material show the presence of a variety of microörganisms, among which the most constant and most abundant is my bacillus *a*. Various anaërobic bacilli are also present, including my

bacillus N. This material injected into guinea-pigs usually produces a fatal result within 24 hours.

June 4, 10 *a. m.*—Injected subcutaneously into guinea-pig, No. 80, 3 minims of fluid obtained at autopsy from stomach of case 22. At 1:30 the animal was observed to be very restless. Found dead the next day at 6 a. m. Extensive subcutaneous œdema, and softening of abdominal muscles.

June 4, 10 *a. m.*—Injected subcutaneously into guinea-pig 84, one-half of a cubic centimetre black vomit from stomach of case 23, obtained at autopsy. Died at 4 p. m. next day. Extensive subcutaneous œdema.

June 29, 1 *p. m.*—Injected subcutaneously into guinea-pig 124, 2 minims of dark grumous material from stomach; case 25, acid reaction. No result.

July 3, 12:30.—Injected subcutaneously into guinea-pig 128, 3 minims of bloody fluid from stomach, case 27. Died at 1:30 July 4. Extensive subcutaneous œdema. Culture from liver several different bacilli (slide 1281). Culture from heart, colon bacillus.

July 16, 7 *a. m.*—Injected subcutaneously into guinea-pig 152, 5 minims of bloody fluid from stomach of case 28. Animal died at 12 m., July 17. Extensive subcutaneous œdema.

TYPICAL "BLACK VOMIT" COLLECTED DURING LIFE AND INJECTED AT ONCE INTO GUINEA-PIGS IS NOT PATHOGENIC.

June 27, 10 *a. m.*—Injected subcutaneously into guinea-pig No. 122, 1 cubic centimetre black vomit from yellow fever case in military hospital (typical black vomit with very acid reaction). Result negative.

July 8, 4 *p. m.*—Injected subcutaneously into guinea-pig 139, 1 cubic centimetre of acid black vomit from case in military hospital, sick 7 days. Result negative.

July 8, 4 *p. m.*—Injected subcutaneously into guinea-pig 140, one-half of a cubic centimetre, acid black vomit (same as in guinea-pig 139). Result negative.

I may remark here that my culture experiments with black vomit have several times given an entirely negative result. (Aërobic cultures in flesh-peptone-gelatine).

MATERIAL OBTAINED FROM THE SMALL INTESTINE OF YELLOW FEVER PATIENTS AT AUTOPSIES MADE SOON AFTER DEATH IS VERY VIRULENT WHEN INJECTED BENEATH THE SKIN OF GUINEA-PIGS.

The contents of the small intestine in yellow fever cases in which death has occurred at the end of 4 to 8 days are usually dark in color and quite viscid from the presence of mucus. The small intestine is lined with this viscid black matter, which shows through its walls and gives it the appearance of being full of a dark substance; but upon cutting it open the quantity is commonly found to be small; sometimes, however, the intestine is filled with a thin black fluid resembling black vomit. No doubt the dark color of the material contained in the intestine, like that of black vomit, is due to the presence of blood pigment changed by the acid secretions, and I am inclined to think that as a rule this pigment comes from the stomach and is not due to intestinal hemorrhage. Although considerable quantities of black vomit are often ejected by the mouth, there can be little doubt that a portion of this fluid passes into the intestine, and that the liquid portion is reab-

sorbed leaving the precipitated blood pigment, mixed with intestinal mucus, as a thin viscid layer upon the surface of the mucous membrane. This is the material which I have collected at my autopsies, and which has served for my culture and inoculation experiments.

Smear preparations stained with fuchsin show that this material contains very numerous and various bacilli.

May 26, 9:30 *a. m.*—Injected subcutaneously into guinea-pig 59, 2 minims material from intestine, case 20. Animal died at 1:30 p. m., May 27. Extensive collection of bloody serum in subcutaneous connective tissue. A few bacilli in liver on direct examination. Spleen rather large; liver normal. A liquefying bacillus obtained in cultures from liver.

May 28, 12 *m.*—Two drops of bloody serum from cellular tissue of above guinea-pig, No. 59, injected beneath the skin of guinea-pig No. 62. The animal died at 6:30 p. m., the same day. Extensive effusion of bloody serum and separation of the skin from subcutaneous tissues over entire abdomen. Various bacilli present in this bloody serum, including one which liquefies gelatine.

May 29, 2:30 *p. m.*—Two minims of bloody serum from cellular tissue of above guinea-pig No. 62, injected beneath the skin of guinea-pig 28. Animal died at 4 p. m., May 31. Extensive subcutaneous œdema with inflammatory thickening of tissues. Cultures from cellular tissue and liver give an actively motile aërobic, liquefying bacillus; slide 1198.

June 1, 10 *a. m.*—One minim of bloody serum from cellular tissue of above guinea pig No. 68, injected beneath the skin of guinea-pig No. 71. Found dead morning of June 3. But little subcutaneous œdema; abdominal viscera normal; bacillus coli commune in gelatine stick culture from blood of heart.

May 27, 12 *m.*—Injected subcutaneously into guinea-pig 61, 2 minims material from intestine, case 21. Found dead at 6 a. m., May 28. Extensive collection of bloody fluid in subcutaneous connective tissue.

June 4, 10 *a. m.*—Injected subcutaneously 2 minims material from intestine, case 22, into guinea-pig 81. Died at 4:30 p. m., June 5. Extensive collection of bloody serum in subcutaneous connective tissue containing bacillus *x*.

June 13, 12 *m.*—Injected subcutaneously 3 minims of viscid mucus, not black, from intestine, case 24, into guinea-pig 102. Died at 8:30, June 14. Bloody serum in connective tissue containing bacillus *h*; this serum has a slightly acid reaction. Abdominal organs normal.

June 14, 10 *a. m.*—Three minims of bloody serum from cellular tissue of above guinea-pig No. 102, injected beneath the skin of guinea-pig No. 105. Died at 7:30, June 15. No subcutaneous œdema. Abdominal viscera normal in appearance. A few bacilli in liver and blood from heart on direct examination. Bacillus *h* recovered in culture from blood of heart.

July 2, 1:30 *p. m.*—Injected subcutaneously into guinea-pig 127, 5 minims of liquid feces from case of yellow fever in civil hospital, eighth day of sickness. Animal died at 12 m., July 5. Liver rather light in color; spleen enlarged.

July 3, 12:30 *p. m.*—Injected subcutaneously into guinea-pig 129, 3 minims bloody fluid from intestine, case 14 (slide 1276). Died at 2 p. m., July 4. Extensive subcutaneous œdema. Cultures from blood of heart and from liver contain bacillus *a*, also a micrococcus; cultures from serum in connective tissue, bacillus *a* and a liquefying bacillus (*k*).

July 4, 2:30 *p. m.*—Injected subcutaneously into guinea-pig 133, 2 minims bloody serum from connective tissue of guinea-pig 129. Result negative.

July 3, 12:30 *p. m.*—Injected subcutaneously into guinea-pig 130, 4 minims of feces from case of yellow fever on German brig in harbor; feces yellow in color liquid, alkaline reaction. Animal found dead at 6 a. m. next morning. Extensive sub-

cutaneous œdema with softening of the muscles. Bacillus *g* recovered from effused serum in cellular tissue of this guinea-pig.

July 6, 12:30 *p. m.*—Injected subcutaneously 1 cubic centimetre culture from heart of guinea-pig 127 (see above). Died July 7, at 2:30 p. m. Same subcutaneous œdema and bloody extravasation; liver rather light in color; uterus intensely congested, escape of bloody fluid from vagina.

July 16, 7 *a. m.*—Injected subcutaneously into guinea-pig 151, 2 minims of material from intestine, case 28, black viscid mucus, acid reaction. Animal recovered; abscess at point of inoculation and in middle of belly.

July 29, 8 *p. m.*—Injected subcutaneously into guinea-pig 168, 3 minims material from intestine, case 29, viscid mucus, not black, acid (slide 1367). Died at 1 p. m., July 30. Extensive collection of bloody serum in subcutaneous tissues and softening of muscles of abdomen. Bacillus N present.

July 30, 3:15 *p. m.*—Injected subcutaneously into guinea-pig 169, 3 minims of bloody serum from cellular tissue of guinea-pig 168. Died at 5 p. m., July 31. Extensive subcutaneous effusion of bloody serum, separation of skin from abdominal walls, and softening of abdominal muscles. Spleen normal, liver rather light in color, contains a few bacilli. Various bacilli in serum from cellular tissue (slide 1372).

July 30, 3:30 *p. m.*—Injected subcutaneously into rabbit 136, 10 minims bloody serum from guinea-pig 168 (bacillus N, etc.). Animal found dead at 5 a. m., July 31.

July 31, 9 *a. m.*—Injected subcutaneously into guinea-pig 171, 3 minims bloody serum from cellular tissue of guinea-pig 169. Died at 9 p. m. the same day. Extensive subcutaneous œdema, small intestine contains a bloody fluid. Bacillus N in effused bloody serum (slide 1376).

July 31, 10:30 *p. m.*—Injected subcutaneously into guinea-pig 173, 3 minims of bloody serum from cellular tissue of guinea-pig 171. Died at 10 a. m., August 1. Extensive separation of skin and softening of subcutaneous tissues, with some bloody effusion. Small intestine hyperæmic and contains a bloody fluid, liver rather soft, gall bladder empty, spleen normal.

August 1, 12 *m.*—Injected subcutaneously into guinea-pig 174, 3 minims bloody serum from cellular tissue of guinea-pig 173. Found dead at 6 a. m. next day. Usual extensive subcutaneous œdema containing bacillus N and other bacilli, spleen large, stomach hyperæmic.

August 1, 12 *m.*—Injected subcutuneously into guinea-pig 175, 4 minims bloody fluid from intestine of guinea-pig 173. Died at 8 a. m., August 2. Extensive softening of tissues and subcutaneous œdema, intense hyperæmia of small intestine, spleen slightly enlarged, liver normal in appearance. Bacillus N in effused bloody serum.

August 9, 10 *p. m.*—Injected subcutaneously into guinea-pig 18, 2 minims from intestine of case 30; viscid mucus, dark in color, slightly acid. Died at 5 p. m. August 10. Extensive subcutaneous œdema containing bacillus N, slide 1413. Spleen somewhat enlarged, liver normal. Bacillus *x* in anaërobic culture from effused serum in cellular tissue.

August 10, 5:30 *p. m.*—Injected subcutaneously into guinea-pig 189, 2 minims of bloody serum from cellular tissue of guinea-pig 182. Died at 3 p. m., August 11. Very extensive subcutaneous œdema, with separation of skin and softening of muscles.

August 11, 3 *p.m.*—Injected subcutaneously into rabbit 162, 5 minims bloody serum from cellular tissue of guinea-pig 184; contains bacillus N, etc. Died at 10:30 a. m., August 15. Abscess in middle of belly, liver dark in color, spleen normal. Liver kept 48 hours in antiseptic wrapping contains bacillus N and other bacilli.

August 10, 11:30 *a. m.*—Injected subcutaneously into guinea-pig 183, 2 minims viscid mucus of a brown color and sightly acid reaction from intestine of case 31. Dead at 6 a. m., August 13. Extensive subcutaneous œdema. Cultures in gelatin from effused serum contain colon bacillus.

August 12, 3 *p. m.*—Injected subcutaneously into guinea-pig 187, 3 minims material

from intestine of case 32, black color, alkaline reaction. Animal had an abscess in middle of belly, but recovered.

August 13, 12:30 *p. m.*—Injected subcutaneously into guinea-pig 188, 2 minims of viscid, black material from intestine case 20. Contains bacillus N. Died at 9:30, August 14. Very extensive subcutaneous œdema with softening of abdominal muscles and separation of skin from abdominal walls. Usual odor.

REMARK: An offensive odor is given off from the necrosed tissues when the abdominal pouch containing bloody serum is opened. The extent of the disorganization of tissue which occurs, often in less than 18 hours, is surprising, and is evidence of the great virulence of this material from the intestine.

August 15, 2 *p. m.*—Injected subcutaneously into rabbit 172, 2 minims material from intestine of case 35 (alkaline). Dead at 6 a. m. next day. Liver dark in color, contains a few bacilli and numerous stained masses (?), slide 1438. No subcutaneous œdema. Spleen normal; bacillus *x* obtained in cultures from liver.

August 19, 7:30.—Injected subcutaneously into guinea-pig 192, 2 minims material from intestine case 36 (acid.) Animal found dead at 6 a. m. next day. The usual subcutaneous œdema, with separation of skin and softening of abdominal muscles.

August 21, 10:30 *p. m.*—Injected subcutaneously into guinea-pig 195, 3 minims viscid black material from intestine case 37. Animal found dead at 6 a. m., August 23. Extensive subcutaneous œdema; contains bacillus N. Softening of muscles and separation of skin, liver rather soft, small intestine contains a bloody fluid. Culture from liver contains bacillus *x*.

August 21, 2 *p. m.*—Injected subcutaneously into guinea-pig 196, 2 minims viscid material, not black, from intestine case 38 (acid reaction). Animal dead at 6 a. m., August 24. Subcutaneous œdema not as pronounced as usual, liver rather light in color, spleen enlarged.

MATERIAL FROM THE INTESTINE DOES NOT ALWAYS KILL GUINEA PIGS AND RABBITS.

June 29, 1 *p. m.*—Injected subcutaneously into guinea-pig 125, 2 minims of material from intestine of case 25. Result negative.

August 19, 7:30 *p. m.*—Injected subcutaneously into rabbit 177, 3 minims material from intestine of case 36 (acid). Result negative.

MATERIAL FROM THE LIVER AND INTESTINE KEPT IN THE COLLECTING BULB FOR TWO WEEKS OR MORE LOSES ITS VIRULENCE.

May 28, 4 *p. m.*—Injected subcutaneously into guinea-pig 65, 2 minims of material from intestine case 17, kept in laboratory for 16 days (alkaline reaction). Result negative.

May 29, 2:30 *p. m.*—Injected subcutaneously into guinea-pig 67, 2 minims of material from intestine case 15, kept in laboratory for 1 month (alkaline reaction). Result negative.

May 30, 3 *p. m.*—Injected subcutaneously into guinea-pig 69, 2 minims of bloody fluid from collecting bulb containing material from liver case 18, in laboratory 2 weeks. Result negative.

May 30, 3 *p. m.*—Injected subcutaneously into guinea-pig 70, 2 minims material from intestine case 18, kept in laboratory 2 weeks, and now decidedly alkaline. Result negative.

COMPARATIVE EXPERIMENTS.

June 6, 10 *a. m.*—Injected subcutaneously into guinea-pig 86, 5 minims material from small intestine, case of heart disease (comparative autopsy No. 7); fluid has a yellow color and slightly acid reaction. Animal found dead next day at 6 a. m. Subcutaneous œdema.

June 8, 12 *m.*—Injected subcutaneously into guinea-pig 92, 3 minims bloody serum from connective tissue of guinea pig 86. Animal died June 11 at 10 a. m. No subcutaneous œdema, abscess in middle of belly, no microörganisms from liver, abdominal viscera normal.

This ended the series, and I have not made further comparative experiments with material from this source. The virulence in the above experiments was decidedly less than in those in which material from the intestine of yellow-fever patients has been injected, inasmuch as the second animal in the series lived for 3 days, and instead of a malignant and rapidly fatal œdema, an abscess was formed in the walls of the abdomen.

It will be seen from the experiments quoted that the virulence of material from the small intestine of yellow-fever cadavers is even greater than is that of liver kept 48 hours in an antiseptic wrapping. The microörganisms encountered in the animals killed by such injections are the same in both cases, a fact which gives support to the inference that the bacilli found in the liver came originally from the intestine. And as the virulence in liver tissue is only developed after the development of these microörganisms in large numbers has occurred, we conclude that it depends upon their presence. The loss of virulence in material from the intestine kept for some time shows that virulence is not due to ordinary putrefactive organisms, but is destroyed by the putrefactive alkaline fermentation which occurs. Most of the microorganisms which I have isolated from this virulent material grow freely in an acid medium, and some of them produce an acid reaction in culture media containing glycerine or sugar. My experiments indicate that the large anaërobic bacillus N is chiefly concerned in the production of the extensive inflammatory œdema resulting from the subcutaneous inoculation of material containing it, and death is no doubt due to this local inflammation rather than to the invasion of the blood by other bacilli associated with this strictly anaërobic bacillus, which is not found in blood from the general circulation or in the parenchyma of the liver and spleen. The bacilli recovered from the blood have been various, but as a rule they have not been numerous. Those found most frequently are facultative anaërobic bacilli, and among them my bacillus *a* and bacillus *x* have been the most constant. Bacillus *xx*, which is very pathogenic for guinea pigs, was obtained in a series of animals, starting from guinea pig No. 102, inoculated with material from the intestine of case 24. But this bacillus, in pure cultures, does not produce the intense inflammatory œdema which results from the injection of a little material from the intestine. The same is true as regards all the rest in the list, with the exception of bacillus N; and my injections of cultures containing this, although they have commonly caused the death of the animal from the local inflammatory œdema, have not proved these culture to be as intensely virulent as is the material from the intestine or from liver which has been kept for 48 hours in an antisep-

tic wrapping. When associated with bacillus *x* the cultures have, however, shown very great virulence, as in the following experiments:

August 12, 1:15 *p. m.*—Injected subcutaneously into guinea-pig 186, 5 minims of an anaërobic culture of bacillus N in glycerine agar (contains also bacillus *x*), animal dying at 1 p. m. next day; killed. Very extensive subcutaneous œdema, with separation of the skin and softening of abdominal muscles; odor like that in animals killed directly by injection of material from intestine. Bacillus N in bloody serum from connective tissue; liver rather light in color.

August 12, 2 *p. m.*—Injected subcutaneously into rabbit 168, 4 minims of anaërobic culture in glycerine agar of bacillus N; contains also bacillus *x* (culture from liver of case 30). Animal died at 7 a. m., August 15. Extensive subcutaneous œdema containing bacillus N and bacillus *x*. Abdominal viscera normal in appearance.

NOTE.—Bacillus *x* alone does not kill guinea-pigs or rabbits when injected subcutaneously. This is shown by the following experiment:

June 13, 4 *p. m.*—Injected subcutaneously into guinea-pig 104, one-half cubic centimetre anaërobic culture of bacillus *x* in glycerine agar. Result negative.

Additional experiments with cultures of these bacilli will be given in connection with the systematic account of the various microörganisms isolated from yellow-fever cadavers.

V.—EXAMINATION OF TISSUES PRESERVED IN ALCOHOL.

In all infectious diseases which have been proved to be due to the presence of a parasitic microörganism in the blood, this organism may be demonstrated in properly stained thin sections of the tissues. In such sections we often obtain cross sections of small blood vessels in which the blood corpuscles are in situ, and in which a stained microorganism, if present, would be very apparent. We also have a satisfactory view of the contents of the capillary vessels of the liver, kidney, brain, etc., in well-prepared sections of these organs. Pathologists, therefore, look upon a careful research, by the methods which have been perfected with this object in view, as of prime importance in any attempt to prove whether a given infectious disease depends upon the presence in the blood of a specific microörganism. Moreover, in certain infectious diseases in which a parasitic microörganism has been proved to be the essential etiological factor this organism is not found, as a rule, in the general blood current, but is present in the tissues especially implicated in the morbid process; *e. g.*, in typhoid fever in the spleen and intestinal glands; in tuberculosis, in the tubercular nodules in the lungs and elsewhere. Failure to find a parasitic organism in blood drawn from the finger is therefore not satisfactory evidence of the absence of a specific germ from the tissues of the organs involved.

As in yellow fever the liver and kidneys give evidence of pathological changes resulting from this disease. I have naturally given special attention to these organs in the researches I have made.

The Havana commission in 1879 made numerous sections of material preserved in alcohol from eighteen cases, and a careful examina-

tion of these sections failed to reveal the presence of any microörganisms; but as more satisfactory methods of staining have since been devised, I have not considered the work done at that time as conclusive in this regard.

I therefore wrote to my friend Dr. Daniel M. Burgess, of Havana, sometime during the summer of 1884, requesting him to obtain for me small pieces of liver, kidney, and stomach from one or more typical cases of yellow fever. I made it an essential condition that the autopsies should be made within an hour, or, at the outside, 2 hours after death, so that there might be no question of post-mortem changes. Small pieces of the organs named were to be put at once into a large quantity of strong alcohol. In compliance with my request, Dr. Burgess obtained and forwarded to me material from two cases, which reached me in good condition, and, upon microscopic examination, the liver and kidneys showed the pathological changes constantly found in the disease in question. During the winter of 1884 I mounted numerous thin sections from this material, stained with various aniline colors. In none of them did I find any microörganisms, except upon the surface of the mucous membrane in sections of the stomach, where various organisms—bacilli and micrococci—were to be seen in properly stained sections. These were, however, only upon the surface, attached to the epithelium, or mingled with a granular débris adhering to the surface of the mucous membrane. In the autumn of 1885, during a visit to Dr. Koch's laboratory in Berlin, I had an opportunity to avail myself of the suggestions and valuable assistance of the master in bacteriology, and again studied the material which Dr. Burgess had sent me from Havana by the various methods of staining considered to be most useful in such a research. At the request of Dr. Koch I was assisted in this research by Dr. Carl Seitz, who was at the time engaged upon his studies of the typhoid bacillus, and was an expert in staining and mounting thin sections of the tissues. Dr. Seitz and myself examined numerous sections of liver and kidney stained by various methods with an entirely negative result, so far as the presence of microörganisms was concerned. After my return to Baltimore, in 1886, I again made numerous sections from the same material, and stained them with Loeffler's alkaline solution of methylene blue, which we had also used in Dr. Koch's laboratory, and with other aniline colors, but without any better success.

Desiring to repeat these researches upon fresh material, I wrote to my friend Dr. Burgess, during my stay in Rio (June and July, 1887), requesting him again to collect pathological material for me from at least four cases of yellow fever, so that after my return to Baltimore I might continue these investigations. As before, this material was to be obtained as soon as possible after death, and to be put at once in strong alcohol. About the 1st of December I received from Dr. Bur-

gess the desired material in good condition, together with the following letter:

HAVANA, *November* 19, 1887.

MY DEAR DOCTOR: I send you, per Dr. Spore, of *City of Washington*, which sails to-day, one box of pathological specimens. * * * You can rely implicitly upon the specimens having been taken from well-diagnosticated yellow-fever cases, at the time post-mortem stated on the bottles. All had, besides the proper temperature curve, irritable stomach, black vomit, highly albuminous urine, eventually in most cases suppression of urine, etc. I saw them repeatedly.

The bottles were marked as follows:

Case No. 1.—Sick from August 14 to 19, 1887. Autopsy 1 hour after death.

Case No. 2.—Died September 23, 1887, at 4:30 a. m. Autopsy 2½ hours after death.

Case No. 3.—Died October 5, 1887, 2:30 a. m. Autopsy 15 minutes after death.

Case No. 4.—Died October 26, 1887, 5:30 a. m. Autopsy 7 o'clock a. m., body still warm (temperature 40° C.).

From the above-described material I have had made a large number of very thin sections, which I have studied by various methods of staining and with objectives of high power, the one-eighteenth and one-twelfth inch hom. ol. im. of Zeiss. I have used especially the alkaline solution of methylene blue of Loeffler; Gram's well-known method, with methyl violet, followed by iodine solution and decolorization with alcohol; the method of Weigert, which is the same as Gram's up to the point of removing the sections from the iodine solution, when they are decolorized and dehydrated with a mixture of two parts of aniline oil to one part of xylol. I have been especially pleased with the last-mentioned method, which gives fine views of the tissue elements and any microörganisms which may be present. I also stained numerous sections with fuchsin in solution with carbolic acid (5 per cent.), or with aniline oil (tubercle stain), and with various other aniline colors.

I think I am safe in asserting that all known pathogenic microörganisms may be stained by one or more of the methods above referred to. Indeed, the alkaline solution of methylene blue is, so far as I know, an agent which stains all organisms of this class, although there are differences as to the rapidity with which they stain and the tenacity with which they retain the color imparted to them.

The result of this research has again been negative so far as the *general* presence of any particular microörganism in the material examined is concerned. But in one case (No. IV) I found in the kidney a minute bacillus, which apparently invaded by preference the glomeruli. It was not found in the capillaries generally, but a certain number of foci were found, some small, as shown in Fig. 6, and involving only a portion of a glomerulus, others involving a whole glomerulus and the tissues immediately surrounding it. The appearance was such as one would expect to see in a case in which solitary bacilli, carried in the first place by the blood current, had effected a lodgment and established a center of infection in tissues already, perhaps, necrotic, and through which the circulation had ceased. The latter supposition seems to be justified by the fact that there were comparatively few of these

foci, whereas if they had been established while the circulation was still going on we would expect to find numerous secondary foci and a certain number of bacilli in the neighboring capillary vessels. Moreover, there was no evidence of inflammatory reaction as a result of this invasion of the tissues by parasitic organisms. I am, therefore, of the opinion that this is some ordinary saprophyte which had effected a lodgment in the kidney, possibly during the last hours of life when the vital resistance of the tissues was slight, or when, as a result of the blood stasis in the organ, local necrosis had already occurred at certain points before death.

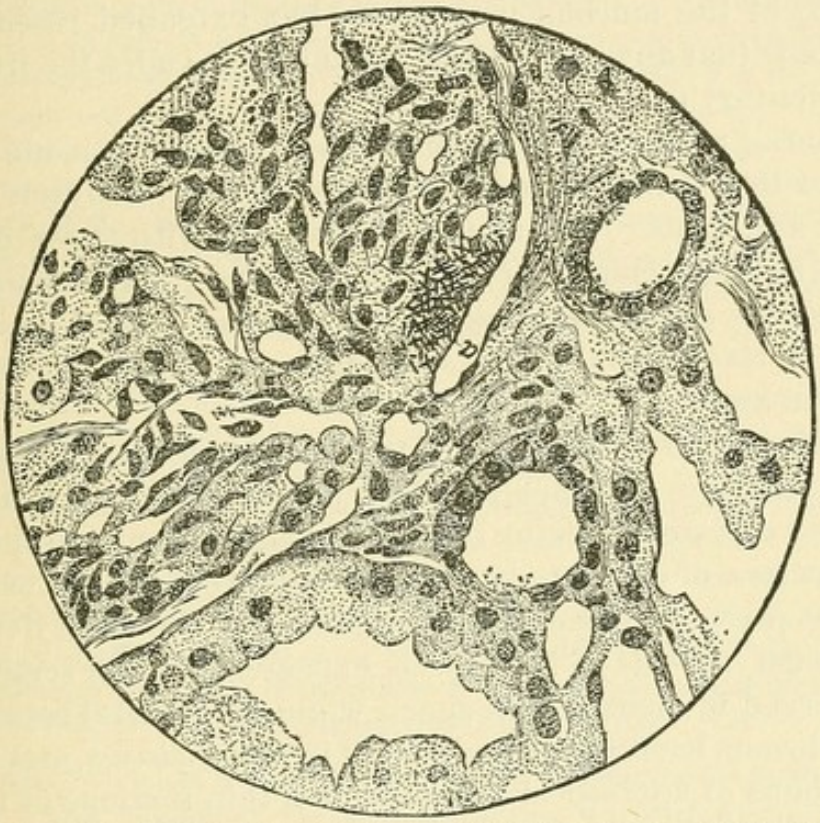


FIG. 6.—Collection of straight bacilli in glomerulus, yellow fever kidney. Material from Havana.

It is quite probable that during the last hours of life a certain number of microörganisms from the intestine succeed in passing through the enfeebled tissues to the interior of the capillaries, and are carried away by the already slowly moving blood stream to distant organs, where they may establish centers of growth even before death occurs, or are at least in position to take possession of the field as soon as the vital spark has been extinguished. In the case in question I believe that the true explanation of the presence of the organisms described is that suggested, for I have not found in the other cases examined any similar collection of bacilli, and can not therefore attach any importance to the observation so far as the etiology of yellow fever is concerned. In Berlin I fell upon a little group of minute, slender bacilli, in a capillary of the liver, and recently I have found a similar group in a preparation of skin from a yellow fever patient. I have also in the course of my extended observations seen two or three groups of micrococci, or of what appeared to be micrococci. But I attach no importance to such

observations. Evidently any organism concerned in the etiology of an infectious disease should be found not occasionally and in certain cases only, but if seen at all by the staining methods adopted it should be found distributed through the organs involved in sufficient numbers to leave no doubt as to its presence, not as an accident, but as a general and constant thing in all cases of the disease under investigation.

The bacillus above described, present in a single case, is, then, the only microörganism found in the material obtained in Havana in 1887 so far as the liver and kidney is concerned. In my stained sections of stomach and intestine I have observed various microörganisms, upon the surface of the mucous membrane, but extended researches have failed to show that any one of these organisms invades the living tissues of the alimentary canal.

The material preserved in alcohol at my autopsies made in Havana in 1888 and 1889 has also been carefully studied by myself and by my laboratory assistants. The results correspond with those obtained by the culture methods employed in the same cases. *In those cases in which my cultures gave a positive result I have, as a rule, found the same microorganisms in thin sections of the same material*—liver, spleen, kidney—preserved in alcohol.

Thus the sections from case 9 (1888) contain numerous bacilli which correspond in their morphology with the colon bacillus which was obtained in my cultures from the blood, liver, and kidney of the same case. The same is true of case 20. In case 14 and in case 33, in which my bacillus N was present in smear preparations from the fresh liver tissue, it is also present, as was to have been expected, in thin sections of the liver preserved in alcohol. In short, while the general result has been negative, various bacilli have been found in certain cases, and in one case (No. 10) groups of micrococci are present in thin sections of the kidney.

In order that this part of the work might be as thorough as possible and free from the reproach of personal bias or imperfect technique, I have had a series of sections made from twenty-five of my Havana autopsies by my friend Dr. James E. Reeves, of Chattanooga, Tenn., and have placed them beside my own sections and those made under my direction by my laboratory assistant, Dr. Emilio Martinez. These slides will be transmitted with my report for permanent preservation in the Army Medical Museum.

Further, I have submitted this entire series of slides to Dr. William T. Councilman, of the Johns Hopkins University, for careful study, and give below his report upon the results of his examinations:

I was requested by Dr. Sternberg to examine the material which he had collected from a large number of cases of yellow fever. This material was collected in Havana and in the South in the epidemic of 1888. Sections which had been made by Dr. Reeves in Chattanooga, and under Dr. Sternberg's direction at the Johns Hopkins University, were carefully examined, and, in addition, the material from thirty autopsies was given me by Dr. Sternberg and further investigated at the pathological laboratory of the Johns Hopkins Hospital. Most of this material was obtained from

fresh autopsies 2 to 12 hours after death and was hardened in alcohol, only three of the cases examined were hardened in Muller's fluid.

One hundred and thirty sections were examined, among them three which Dr. Sternberg had obtained from Dr. Freire in Brazil. These sections were stained with methylene blue, gentian violet, Bismarck brown, and with the Gram and Weigert methods. The sections which were given Dr. Sternberg by Dr. Freire in Brazil were stained red, probably with fuchsin. Of these specimens it is not necessary to say much. It was most impossible to say from what tissue they were made, and to have recognized any organisms in the precipitate of staining fluid and other débris would have been impossible. The sections made by Dr. Reeves and those under Dr. Sternberg's direction were in general good, particularly the latter. It is probable that these sections show clearly all the bacteria which are contained in the tissues, for other more complicated methods of staining gave the same results as to bacteria. All of the bacteria found were stained with the simplest methods. Bacteria of some sort were found in 28 of the 130 sections examined; of these 18 were sections of the liver, 8 of the kidney, and 1 each of stomach and lymph gland. There was nothing in their form or relation to the tissue that would lead one to suppose that their presence was other than accidental. In no case could any connection be shown between their presence and the essential lesions of the disease. There were both micrococci and bacilli, in some cases arranged in groups, in others they were single or in indefinite masses. In no case was their any lesion in the surrounding tissue which could be attributed to their presence. Among the bacilli were some which agreed in form with the colon bacillus.

The micrococci were in the form of the well-known emboli, and were found in the blood vessels of the liver and kidneys; in the latter generally in the glomeruli. In but one case were bacilli found in the tubules of the kidney.

Five sections of stomach were examined, but nothing characteristic found in these. In one of these sections there was some evidence of gastritis shown, by the presence of leucocytes in and between the epithelial cells, and below the epithelium some small cell infiltration. The epithelium was in general well preserved.

The sections of intestine, spleen, and lymphatic glands were perfectly normal. In one of the sections of lymphatic glands there were numerous masses of short bacilli, which were also found in the other organs from the same case. (Case 9.)

The following is a list of the slides which I propose to transmit with this report. The result of Dr. Councilman's careful examination for bacteria will be given in parentheses in every case where this result was positive. No remark is made when the result was negative.

When no remark is made with reference to the method of staining employed, it will be understood that Loeffler's alkaline solution of methylene blue was the staining agent.

No. 1. Kidney of guinea-pig which died July 23, 1887. Mounted by Dr. Domingos Freire in his laboratory, and said by him to contain his yellow-fever microbe.

2. Section of kidney of guinea-pig killed on the 16th of July, 1887. Mounted by Dr. Freire, and said by him to contain his micrococcus.

3. Liver of guinea-pig which died July 23, 1887. Mounted by Dr. Freire, and said by him to contain his micrococcus.

NOTE.—These slides are the only ones given to me by Dr. Freire, and are placed in this series for permanent preservation and as evidence of his microscopical technique.

4. Section of yellow fever kidney, made in the laboratory of Dr. Lacerda, in Rio de Janeiro, by Dr. Araujo Goes. Contains the bacillus of Lacerda and Babes.

No. 5. Yellow fever kidney. Bismark brown staining; section made by Dr. Sternberg in 1884.

6. Yellow fever kidney; methylene blue staining. Section made by Dr. Sternberg in 1884. Shows numerous plasma cells.

7. Blood of yellow fever case in Misericordia Hospital, Rio de Janeiro, 1887. Fourth day of sickness; kept in capillary tube for 5 days and then stained with fuchsin by Dr. Sternberg.

8. Blood of yellow fever patient in small-pox hospital, Rio de Janeiro, 1887. Third day of sickness; stained with fuchsin by Sternberg.

9. Blood from same case as No. 8, stained with osmic acid and fuchsin.

10. Blood from yellow fever case in Misericordia Hospital, Rio de Janeiro, 1887. Fourth day of sickness. Collected and stained by Dr. Sternberg.

11. Blood of case of yellow fever in small-pox hospital Rio de Janeiro, 1887. Third day of sickness; collected and stained by Dr. Sternberg.

12. Yellow fever liver; section made in Dr. Lacerda's laboratory, June 26, 1887, by Dr. Araujo Goes; stained with methylene blue by Dr. Sternberg to show the bacillus of Lacerda and Babes. ("Bacilli" C.)

13. Yellow fever blood collected by Dr. Sternberg in Havana in 1879. Showing crystals of hæmatin.

14. Slide from Dr. Carmona's laboratory in the City of Mexico. Stained and mounted by Dr. A. Gavino. Showing bacilli obtained by cultivation from yellow fever urine.

15. Yellow fever blood collected and dried on slide in Rio de Janeiro. Stained with eosin. Showing eosin "philine granules."

16. Sections of intestine, case 3, 1887. Stained by Gram's method. Numerous amorphous stained masses in intestinal mucus (?.)

17. Sections of stomach, case 1, 1887. Stained with carbol fuchsin.

18. Sections of stomach, case 1, 1887. Stained with methylene blue. ("A few bacteria scattered over the surface" C.)

19. Sections of stomach, case 4, 1887. Stained by Weigert's method.

20. Sections of stomach, case 3, 1887. Stained by Gram's method.

21. Sections of stomach, case 4, 1887. Stained by Weigert's method.

22. Section of stomach, case 2, 1887. Stained with methylene blue.

23. Liver, case 1, 1887. Stained with Loeffler's solution of methylene blue. ("Bacilli" C.)

24. Kidney, case 1, 1887.

25. Kidney, case 1, 1887. Weigert's method.

26. Kidney, case 2, 1887.

27. Liver, case 2, 1887.

28. Liver, case 2, 1887. Aniline fuchsin.

29. Kidney, case 2, 1887. Carbol fuchsin.

30. Kidney, case 3, 1887. Aniline fuchsin.

31. Kidney, case 3, 1887.

32. Kidney, case 3, 1887. ("Bacilli in epithelium" C.)

33. Liver, case 3, 1887.

34. Liver, case 4, 1887.

35. Kidney, case 4, 1887.

36. Kidney, case 4, 1887.

37. Kidney, case 4, 1887. Aniline fuchsin.

38. Spleen, case 2, 1887.

39. Liver, case 2, 1887. ("Leucocytic invasion and bacteria" C.)

40. Kidney, case 2, 1887.

41. Liver, case 3, 1887.

42. Liver, case 3, 1887.

43. Liver, case 3, 1887.

No. 44. Intestine, case 3, 1887.
45. Stomach, case 3, 1887.
46. Kidney, case 4, 1887.
47. Kidney, case 1, 1888.
48. Liver, case 1, 1888.
49. Kidney, case 1, 1888.
50. Spleen, case 2, 1888.
51. Kidney, case 2, 1888.
52. Kidney, case 2, 1888.
53. Liver, case 3, 1888.
54. Kidney, case 3, 1888.
55. Kidney, case 3, 1888.
56. Liver, case 3, 1888.
57. Kidney, case 4, 1888.
58. Liver, case 4, 1888. ("Shows beautiful inflammation, bacilli," C.)
59. Liver, case 4, 1888.
60. Kidney, case 4, 1888.
61. Liver, case 9, 1888.
62. Kidney, case 9, 1888.
63. Liver, case 9, 1888.
64. Kidney, case 9, 1888. ("Bacilli," C.)
65. Mesenteric gland, case 9, 1888. ("Bacilli," C.)

REMARKS.—In case 9, 1888, I obtained the colon bacillus in my cultures from the blood, liver, and kidney. The bacilli present in stained sections correspond with this bacillus in their morphology, and are no doubt the same, *i. e.*, the *bacterium coli commune* of Escherich.

66. Kidney, case 10, 1888. ("Micrococci in vessels," C.)
67. Kidney, case 10, 1888.
68. Kidney, case 10, 1888. ("Cocci in vessels and glomeruli," C.)

REMARKS.—Case 10, 1888, is the only one in the whole series in which micrococci have been present in any considerable number. In this case they occur in the capillaries of the kidney, in the form of "emboli" of considerable size.

69. Liver, case 10, 1888.
70. Kidney, case 1, 1889.
71. Spleen, case 1, 1889. ("Short bacilli," C.)
72. Liver, case 1, 1889. ("Bacilli, short and thick," C.)

REMARKS.—The bacillus in this case is my bacillus N, which was found in smear preparations from the fresh liver and spleen, and obtained in anaërobic cultures from the same case.

73. Kidney, case 1, 1889.
74. Liver, case 1, 1889.
75. Mesenteric gland, case 1, 1889.
76. Kidney, case 2, 1889.
77. Liver, case 2, 1889.
78. Kidney, case 2, 1889.
79. Liver, case 2, 1889.
80. Liver, case 3, 1889.
81. Kidney, case 3, 1889.
82. Liver, case 3, 1889.
83. Kidney, case 4, 1889.
84. Liver, case 4, 1889.
85. Kidney, case 4, 1889.
86. Liver, case 4, 1889.
87. Liver, case 5, 1889. ("Short bacilli in small numbers in capillaries, in pairs and in short chains," C.)

No. 88. Kidney, case 5, 1889. ("In vessels and glomeruli, short bacilli in pairs and groups," C.)

89. Liver, case 5, 1889. ("Bacilli," C.)

90. Kidney, case 5, 1889. ("Bacilli," C.)

REMARKS.—Dr. Councilman's notes show that he found bacilli in all of the sections from this case. Turning to my notes relating to the examination of fresh material from the same case I find the following, "Case 18" (No. 5 of 1889): "Sick 5 days; autopsy 2 hours after death. Direct examination of blood negative; of liver a few small oval bacilli in pairs. Aërobic gelatine Esmarch tube from blood contains a few colonies of bacillus *a*. Anaërobic agar Esmarch tube from blood contains numerous colonies of bacillus *a*, and of a short bacillus in chains. Anaërobic agar Esmarch tube from kidney contains numerous colonies bacillus *w*, the same from liver."

91. Liver, case 7, 1889.

92. Liver, case 8, 1889. ("Small groups of short bacilli, and in one place micrococci in pairs." C.)

REMARKS.—In this case my bacillus N was obtained in anaërobic cultures from the liver, and this is the bacillus present in the section examined by Dr. Councilman.

93. Liver, case 11, 1889.

94. Liver, case 11, 1889.

95. Kidney, case 11, 1889.

96. Kindey, case 11, 1889.

97. Liver, case 14, 1889.

98. Liver, case 14, 1889.

99. Liver, case 14, 1889. ("Bacilli, short and thick in capilla ies," C.)

100. Liver, case 16, 1889. ("Same as 90," C.)

REMARKS.—In this case my notes show that numerous bacilli were found in a smear preparation from the fresh liver tissue, and that both bacillus *a* and bacillus *x* were obtained in my cultures from fresh liver tissue.

101. Liver, case 17, 1889.

102. Liver, case 17, 1889. ("A few long slender single bacilli, and groups of short bacilli in capillaries," C.)

REMARKS.—My cultures from the liver of this case gave the following result: "Aërobic cultures from liver contain both bacillus *a* and bacillus *x*. Anaërobic cultures from liver bacillus N and bacillus O."

103. Liver, case 18, 1889.

104. Liver, case 18, 1889. ("One group of rather large organisms," C.)

105. Liver, case 19, 1889.

106. Liver, case 19, 1889.

107. Liver, case 20, 1889. ("Short bacilli in groups." C.)

108. Liver, case 20, 1889. ("Bacilli in groups." C.)

REMARKS.—In this case my bacillus N was present in a smear preparation from the fresh liver tissue, and "numerous colonies of bacillus *a* were obtained in gelatine Esmarch tubes from the liver."

109. Liver, case 21, 1889.

110. Liver, case 21, 1889.

111. Liver, case 22, 1889.

112. Liver, case 22, 1889.

113. Liver, case 24, 1889.

114. Liver, case 24, 1889.

115. Liver, case 25, 1889.

116. Liver, case 25, 1889.
117. Liver, case 26, 1889.
118. Liver, case 27, 1889.
119. Liver, case 28, 1889.
120. Liver, case 29, 1889.
121. Kidney from Dr. Lacerdo's laboratory, containing bacillus of Babes and Lacerdo. ("Large numbers of short bacilli in chains," C.)

SMEAR PREPARATIONS.

122. Kidney kept 48 hours in an antiseptic wrapping. Case 1, Havana, 1888.
123. Liver kept 48 hours in an antiseptic wrapping. Case 8, Havana, 1889.
124. Liver kept 48 hours in an antiseptic wrapping. Case 13, Havana, 1889.
125. Liver kept 48 hours in an antiseptic wrapping. Case 29, Havana, 1889.
126. Liver, case 1, Havana, 1888; kept for 48 hours in antiseptic wrapping.
127. Liver, case 2, Decatur, 1888; kept for 48 hours in antiseptic wrapping.
128. Liver, case 10, Havana, 1888; kept for 48 hours in antiseptic wrapping.
129. Liver, case 1, Havana, 1889; kept for 48 hours in antiseptic wrapping.
130. Liver, case 4, Havana, 1889; kept for 48 hours in antiseptic wrapping.
131. Liver, case 7, Havana, 1889; kept for 48 hours in antiseptic wrapping.
132. Liver, case 14, Havana, 1889; kept for 48 hours in antiseptic wrapping.
133. Liver, case 21, Havana, 1889; kept for 48 hours in antiseptic wrapping.
134. Liver, case 29, Havana, 1889; kept for 48 hours in antiseptic wrapping.
135. Contents of intestine, case 1, Havana, 1888.
136. Contents of intestine, case 2, Decatur, 1888.
137. Contents of intestine, case 3, Decatur, 1888.
138. Contents of intestine, case 1, Decatur, 1888.
139. Contents of intestine, case 2, Decatur, 1888.
140. Feces, case 1, Decatur, 1888, 48 hours sick.
141. Feces, case 2, Decatur, 60 hours sick.
142. Feces, case 5, Decatur, 36 hours sick.
143. Feces, case 6, Decatur, 48 hours sick.
144. Feces, case 7, Decatur, 4 hours sick.
145. Feces, case 8, Decatur, 24 hours sick.
146. Feces, case 9, Decatur, 24 hours sick.
147. Contents of intestine, case 4, Havana, 1889.
148. Contents of intestine, case 5, Havana, 1889.
149. Contents of intestine, case 9, Havana, 1889.
150. Contents of intestine, case 11, Havana, 1889.
151. Contents of intestine, case 8, Havana, 1889.
152. Contents of intestine, case 15, Havana, 1889.
153. Contents of intestine, case 22, Havana, 1889.
154. Contents of stomach, case 2, Havana, 1889.
155. Contents of stomach, case 4, Havana, 1889.
156. Contents of stomach, case 9, Havana, 1889.
157. Contents of stomach, case 11, Havana, 1889.
158. Contents of stomach, case 13, Havana, 1889.
159. Contents of stomach, case 14, Havana, 1889.
160. Black vomit, typical, acid reaction, seventh day of sickness, used to inoculate guinea pig 105.
161. Black vomit, typical, acid reaction, used to inoculate guinea pig 88.
162 to No. 180. Sections of yellow-fever liver and kidney, series of 1889. Mounted by Dr. William T. Councilman. Double stained with eosine and methyline blue.

PATHOLOGICAL ANATOMY AND HISTOLOGY.

I shall introduce here that portion of my article contributed to "Wood's Handbook of the Medical Sciences" which relates to the pathology of the disease:

The exterior of the body of an individual who has recently succumbed to yellow fever presents an appearance which is quite characteristic. The icteric color of the skin, although often not noticeable during the last hours of life, is developed in a large majority of the cases very soon after death. The color is that which is seen to follow a bruise in which there has been an effusion of blood, and the origin of the pigment is no doubt the same. The icterus from bile pigments, which occurs not infrequently during convalescence, and which may be seen in cases fatal from a relapse or at a late period of the disease, gives a uniform saffron-yellow color to the surface of the body and conjunctivæ. In the icteric discoloration of which we speak at present the color is not so intense and not so uniformly distributed. The depending portions of the body and especially those subjected to pressure have a deeper coloration, and are more or less livid and mottled from hypostatic congestion. Dutrouleau has shown that this ecchymotic appearance of the back is due to position and pressure by placing the body upon the abdomen or side. In this case it is still the most dependent part which shows the livid marbled appearance referred to. The face and hands frequently appear cyanosed, or the face may be livid and turgescent, like that of one recently drowned. A little stream of black vomit is frequently seen trickling down from the corners of the mouth, or a similar fluid may escape from the nostrils, and the lips and gums may be soiled with dark blood which has oozed from them.

Cadaveric rigidity is quickly established and well marked.

The appearances observed upon removing the calvarium are usually those of hyperæmia of the *brain* and its *meninges*. The pia mater is almost always congested and the vessels of the brain are abnormally full of blood; the pons and medulla are especially in a condition of hyperæmia. There is more or less effusion into the subarachnoid space and in the ventricles; this is sometimes turbid and has a yellow color. The surface of the brain presents sometimes little hæmorrhagic points, and its substance, like the tissues of the body generally, has a more or less pronounced yellow tinge.

Dr. Schmidt, of New Orleans, has described certain pathological changes in the *sympathetic ganglia* which he believes to be important. In a majority of the cases examined by him the minute blood vessels of the ganglia were found to be filled with blood corpuscles. "In two cases the ganglionic bodies of the first thoracic as well as of the semilunar ganglion had most obviously undergone degeneration. In the greater part of these ganglion cells the nuclei had entirely disappeared, leaving no other trace but their nucleoli, which were very distinct; in the rest very faint outlines of the nuclei could still be observed. The bodies of all these ganglion cells presented an indistinct appearance, and were characterized by a peculiar fatty luster, even observed in specimens mounted in Canada balsam."

Usually the *lungs* present no evidence of pathological changes; occasionally they are congested and contain hemorrhagic infarctions.

The fluid in the *pericardium* is often increased in amount and has a yellow color; in rare cases it contains blood. The *heart* is commonly paler than normal, and according to some authors is often soft and friable, owing to fatty degeneration of its muscular tissue (Riddel Schmidt). This is denied by others (Crevaux, Guiteras, Gama Lobo). Woodward, who examined the material brought back by the Havana Commission of 1879, agrees with Guiteras that his sections did not present any decided evidence of fatty degeneration, but remarks: "This by no means proves that it does not occur in certain cases, perhaps in certain groups of cases."

Several of the most competent observers agree that there is a fatty degeneration of the walls of the small *blood vessels* and *capillaries* of the various organs, and account in this way for the hæmorrhagic tendency of the disease. This is certainly a more rational explanation than the one so frequently offered, viz, that the hæmorrhages are due to the disorganized and diffluent condition of the blood. A moment's reflection should show that this explanation is insufficient, and that the blood, however diffluent, can not escape so long as the vessels are intact. Ecchymotic points or hæmorrhagic infractions are sometimes found upon the surface or in the substance of the heart, as well as in other muscles of the body and the organs generally. The cavities of the right side of the heart usually contain soft coagula or dark-colored fluid blood, and the right ventricle sometimes contains a more or less decolorized fibrinous clot.

The *blood* in yellow fever has been described by many observers as "completely disorganized" as to its histological elements. This is a mistake, as is shown by the numerous photomicrographs made by the writer while in Havana, in 1879, from the blood of cases near a fatal termination. Both the red and the white corpuscles retain their normal appearance, and I have frequently seen the leucocytes undergoing their characteristic movements, even after 24 hours, in blood which had been preserved in culture cells. In the days of bleeding numerous observers mentioned the fact that the blood does not coagulate readily, or it forms a soft, loose coagulum from which the serum does not separate. The same is true of the blood in the heart and large vessels after death; it is fluid and dark colored. Although there is no general destruction of the red corpuscles, it is probable that a considerable number of these elements perish in severe cases, for the serum contains free hæmoglobin, which gives it a yellow color even as early as the third or fourth day. This yellow color is seen in the serum obtained from the application of blisters to the surface, and in blood drawn for microscopical examination. In blood obtained near the termination of a fatal case, and in post-mortem blood, this color is very pronounced, and increases in intensity by further solution of the hæmoglobin when the specimen is kept for a time; in the meantime the blood disks become paler than normal. In specimens kept in culture cells I have observed the formation of beautiful crystals of hæmatoidin. This passing of the hæmoglobin into the serum, which is no doubt the cause of the yellow discoloration of the tissues in yellow fever, has been ascribed to the presence of bile. This view is not sustained by the chemical researches of Cunisset, a recent French author, who has arrived at the following conclusions:

"Yellow fever is not a poisoning by the bile; at the outset of the malady the biliary pigments are rarely found in the blood or in the urine. They appear generally only during the second period, and in a great number of cases they are not to be found at all.

"The biliary salts, of which the powerful action of 'deglobulization' admitted by certain authors might explain the disorders which the malady presents, do not exist either in the matters vomited or in the urine or in the blood except in certain cases in very feeble quantity. In view of the profound alterations of the liver, this absence of the biliary salts is easily understood, and the defective depuration of the blood is to be looked upon as a complication rather than a determining cause of the malady."

Schmidt says: "The icterus in yellow fever is not owing to the presence of bile in the blood, as is believed by a large number of physicians, but to the presence of free hæmoglobin, and represents in truth the so-called 'hæmatogenous' jaundice. A 'hepatogenous' jaundice can not take place, as the larger as well as the smaller hepatic ducts are found perfectly open, and as the secretion of bile during the disease, in the majority of cases, is rather diminished, or even suspended."

The white blood corpuscles have seemed to the writer to be rather reduced in number toward the end of fatal cases. In specimens of blood mounted dry for microscopical examination they are frequently seen to contain one or more highly refractive granules, the exact nature of which has not been determined, but which I suppose to

be fat. There are no microörganisms present in the blood of yellow fever demonstrable by the usual methods of staining and microscopical examination, or by cultivation in the media commonly employed by bacteriologists.

The most important pathological changes in yellow fever are found in the organs contained in the cavity of the abdomen. The mucous membrane of the *stomach* is always found to be more or less hyperæmic; the congestion is commonly not general, but is confined to smaller or larger spots or districts, in which it is observed to proceed from one or more centers. From these centers it extends or radiates in a lesser degree, either gradually to be lost or to pass over to another congested district. It is owing to this peculiarity of the congestion that it presents no uniformity of character, but is observed to spread irregularly over larger or smaller portions of the membrane (Schmidt). In addition to these congested patches, there are often to be seen small red patches resembling ecchymoses, but which, according to Schmidt, consist of "an unbroken network of minute vessels congested with blood and identical with that network of large capillaries which surrounds the aperture of the gastric glands." Crevaux believes that a fatty degeneration of the cells which line the gastric glands and the capillaries of the mucous membrane is the most important lesion here. Other authors speak of an acute gastric catarrh as the process indicated by the appearance and histological examination of the mucous membrane; others again deny that there is any inflammation. My own examination of thin sections stained in various ways shows that in a certain proportion of the cases there is evidence of inflammation, as shown by the presence of an unusual number of leucocytes in the submucous coat.

It has been asserted that the epithelium of the stomach undergoes fatty degeneration. This is denied by Schmidt. The dark fluid so often ejected during the last hours of life known as *black vomit* is almost always present in the stomach in greater or less amount after death. There is no question that the dark color is due to the presence of blood pigment, more or less changed by the acid secretions of the stomach. The best authorities everywhere are in accord on this point, which has, however, recently been called in question by Carmona, Freire, and Gibier. These gentlemen suppose the black pigment to be a product produced by the vital processes of a specific microörganism. The two first-named authors base their opinion, that that the color is not due to blood pigment, upon the negative results of their spectroscopic experiments. Dantec remarks, with reference to this: "Everyone knows that the derivatives of hæmoglobin (hemapheine, hematine, etc.) are insoluble in water; it is then not astonishing that Messrs. Domingos, Freire, and Carmona have not obtained any spectroscopic result, since the first condition is to examine the body in a liquid state. Upon dissolving the black matter with acidified alcohol, we have always obtained positive results."

The author just quoted has repeated an experiment often made in this country, at a time when the question was whether the vomited matter consisted of "black bile" or derived its color from the blood. By adding some drops of hydrochloric acid to blood diluted with water, Dantec produced an "artificial black vomit," which resembled exactly that ejected from the stomach of yellow-fever patients.

The small *intestine* commonly contains more or less black matter, either fluid and resembling that found in the stomach, or mixed with mucus and smeared over the mucous coating, especially of the ileum. This, no doubt, comes partly from the stomach, but in other cases is due to passive hemorrhage from the mucous membrane of the intestine itself. This membrane presents arborescent patches of congestion, or portions of the canal may be uniformly red from hyperæmia of the mucous coat; the color varies from pale red to a reddish brown, and is usually more marked in the lower portion of the ileum than elsewhere. The large intestine occasionally presents similar arborescent patches of congestion, but usually it has a normal appearance. Finally, we may say that the attention of pathologists has heretofore been so largely taken up with the pathological histology of the organs which present the most notable changes—liver and kidney—that the histology of the alimentary canal has been

somewhat neglected, and further researches in this direction are desirable upon material obtained at the earliest possible moment after death.

The appearance of the liver in yellow fever is characteristic, at least so far as acute febrile diseases are concerned. Usually it contains less blood than in the normal con-

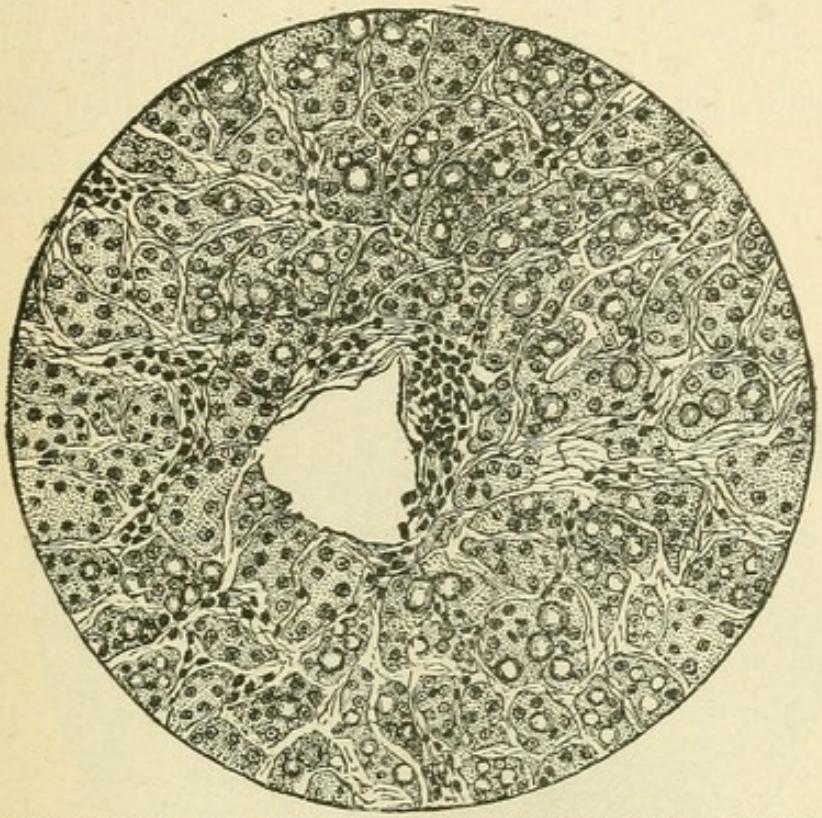


FIG. 7.—Fatty infiltration of the liver cells, with a moderate amount of small-celled infiltration, in a case of yellow fever. Material from Havana.

dition, and is of a pale yellow or brownish yellow color, similar to that of new leather in its various shades; occasionally it is gorged with blood, and livid, deep blue, or dark purple in color. In the victims of chronic alcoholism, it often presents the nutmeg appearance of cirrhosis. The dimensions do not differ materially from the normal, but the consistence is modified by the fatty change, which gives the characteristic color, and the parenchyma is easily torn and more or less friable. On section it is found to be drier than in the normal state, except in the comparatively few cases in which it is hyperæmic; these are, as a rule, cases which have proved fatal at a very early period—a fact which indicates that there is a stage of congestion antedating that of anæmia and fatty degeneration. According to Crevaux, this congestion is located especially in the portal radicles surrounding the lobules, and is attended with œdema of the interlobular connective tissue. Whether this primary congestion is a constant phenomenon or not, it is certain that in a majority of the autopsies the liver is found to be anæmic, and to present more or less evidence of fatty change in the hepatic cells. This is not, however, a uniform process, but areas of greater or less extent are seen, in which the cells are infiltrated with fat globules, as seen in Fig. —; while in other places the cells appear normal. The fatty cells contain one or several fat globules of varying dimensions, and the protoplasm is reduced in quantity according to the extent of this fatty change. The nuclei very often remain intact in the cells infiltrated with fat, but according to Schmidt "a great number of the nuclei also undergo fatty degeneration." Often a collar of normal cells remains about the central vein, while those cells nearer the periphery of the lobule contain numerous fat globules.

In two of the livers brought back from Havana by the Yellow Fever Commission of 1879, in which there was evidence of cirrhosis, Woodward found that, in addition

to the fatty change described, "an abundant infiltration of cells resembling leucocytes was observed, not merely in the abnormally developed interlobular connective tissue, but also in the parenchyma of the lobules." I have also found an infiltration of leucocytes in two out of four cases, which I have recently studied with care. In one, from which Fig. — was drawn, it was attended with the usual fatty change. In the other it is far more pronounced, and the liver cells to a considerable extent are atrophied, and have lost both their nucleus and their protoplasm, indeed a veritable necrosis of the cells has taken place (see Fig. 8.) This, however, is quite an exceptional picture, and probably represents a complication rather than an exag-

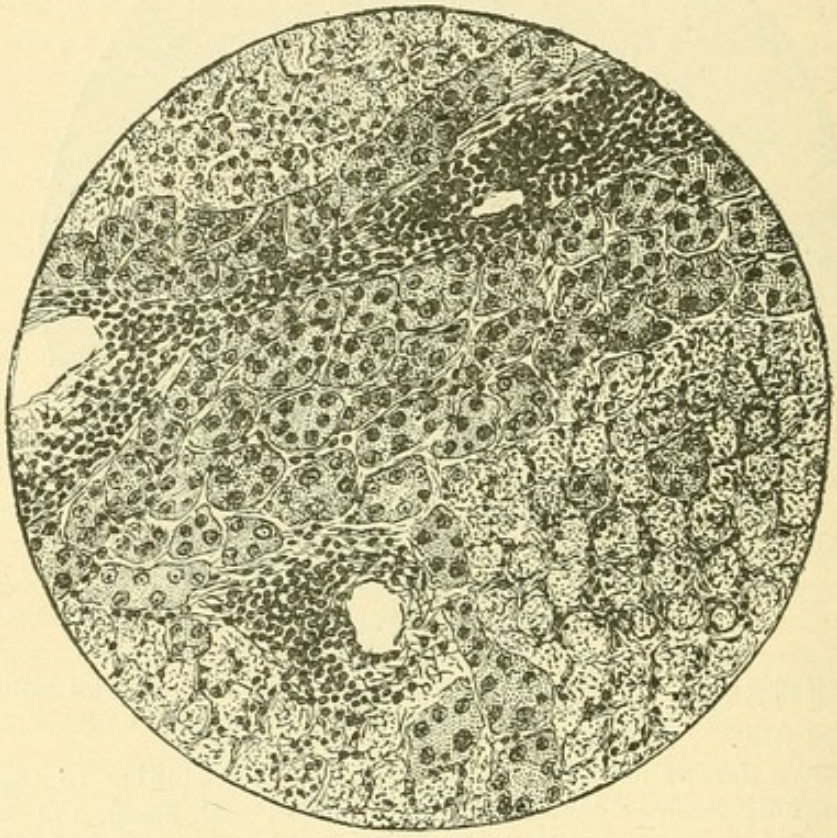


FIG. 8.—Acute hepatitis, with necrosis of the liver cells (a rare condition). From a case of yellow fever. Havana, 1887.

gerated degree of an inflammation common to the disease. Schmidt states that he has never observed any evidence of inflammation in the liver of yellow fever.

The *kidneys* are also the seat of important pathological changes in the disease under consideration. This consists essentially in a parenchymatous nephritis. Externally no material change is noted in the organs. They do not vary greatly from the normal size, and usually are normal in appearance. When the attack has been brief, however, they may be hyperæmic and of a deep red color. Crevaux believes that the changes found in the parenchyma of the organ are usually preceded by a stage of congestion; Schmidt agrees with him, and states that he has met with a limited number of cases in which death had occurred during this stage of hyperæmia. Ecchymosed spots and hemorrhagic foci are frequently seen beneath the capsule or in the cortical substance. In the latter situation little globular hemorrhagic points, the size of a pin's head, have been observed which, upon examination, proved to be the distended capsules of the glomeruli. The change in the renal epithelium consists in a cloudy swelling, followed by fatty degeneration and desquamation. Every grade of change may be seen in the same section, from a slight degree of cloudy swelling to complete disorganization and desquamation of the cells. Whole bundles of tubes may often be seen which have been stripped of their epithelium and are entirely empty. In thin sections the lumen of the tubules is seen in places to be filled with infarctions of various appearance. Some are homogeneous and translucent, and it

may be more or less colored with blood pigment; these are composed of an albuminous material. Other infarctions are formed of the granular débris of the renal epithelium; or we may have a mixture of the granular and albuminous material, in which case the latter forms a matrix in which the granules are imbedded. These infarctions correspond with the casts found in the urine; those of a granular character are most abundant, and in them the granules often have a fatty appearance. There are also accumulations which differ from these in the fact that they are deeply stained by the aniline colors. They seem to be made up of the nuclei of the cells, sometimes intact, although swollen and compressed; more commonly massed together, or broken into regular fragments. At least this seems to be the most probable interpretation of those infarctions which are stained by nuclear staining agents.

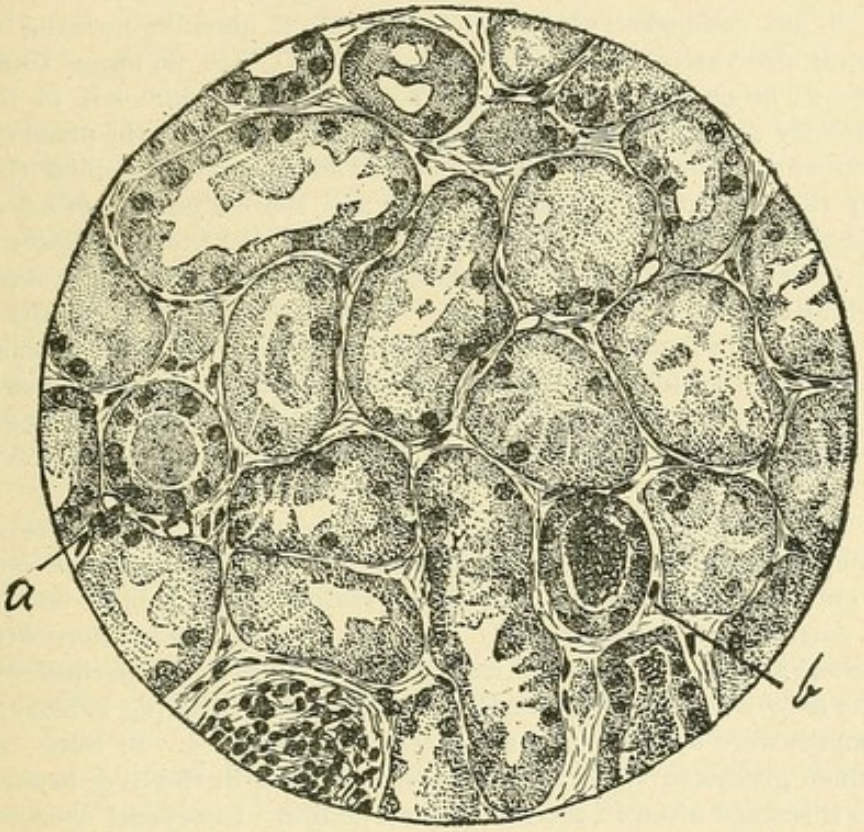


FIG. 9.—Acute parenchymatous nephritis. *a*, hyaline; *b*, granular infarction. From a case of yellow fever. Havana, 1887.

The supra-renal bodies, according to Schmidt, undergo pathological changes similar to those observed in other organs, "consisting in the infiltration of fat and hæmoglobin, derived from the blood, and preceded by hyperæmia of the organ. * * * The infiltration or extravasation of hæmoglobin absorbed by the cells is greater and more general here than has been observed in any other organ. Almost in every case examined it involved the whole medullary substance, from which it extended into the inner and middle layer, sometimes even into portions of the outer layer of the cortical substance. The degree of this pigmented infiltration is sufficiently great to be always distinguished by its brown color in sections of fresh specimens; in some cases, even, it appears dark brown.

The *spleen* does not undergo any marked alteration in yellow fever.

Dr. William T. Councilman has given me the following report upon the pathological histology of the disease, as shown by the series of slides submitted to him for examination:

REPORT OF DR. WILLIAM T. COUNCILMAN.

The most interesting results were obtained from the examination of the liver. It has long been held that fatty degeneration of this organ was one of the most charac-

teristic lesions of yellow fever, and it was found to a greater or less extent in all of the sections examined. It varied greatly in intensity in the different cases; in some comparatively large areas of liver tissue, which showed very little degeneration, were found, in others only here and there a few normal liver cells were seen. This lesion, however, does not seem to me to be the most important one of the organ. When sections of the liver are deeply stained with eosin and subsequently with a nuclear stain, either hæmatoxylin or methyline blue, a very peculiar appearance results. When such sections are examined with a low power the liver cells are found to be stained a faint reddish blue or purple color, the nuclei being a deep blue or purple. Among the liver cells or in place of them a great number of bodies stained intensely red with the eosin are found when examined with a high power. These bodies are found to differ entirely from the liver cells. They are sharply circumscribed, are highly refractive, and are composed of a perfectly hyaline mass containing numerous vacuoles. Their size varies greatly; in some cases they are no larger than a lencocyte, in others as large as two liver cells. They are found inclosed in liver cells, otherwise perfectly normal, and in some cases they entirely take the place of these in the liver beam-work between the capillaries. In some cases examined they apparently made up the mass of the tissue, only here and there a portion of a liver cell or a nucleus of such being seen. Sometimes, especially where the liver tissue was most scanty, along with these definite circumscribed masses more or less granular material was found which stained in the same way. These bodies were generally round or more or less irregular in form. In some of the liver cells small hyaline masses staining in the same way were found which were not so sharply circumscribed as the larger bodies. They were found most abundantly in the cases where the fatty degeneration was most extreme, but the most striking pictures were obtained where the liver was least altered.

In a few instances liver cells were found which only differed from the normal in being more coarsely granular, the granules staining with eosin but not so distinctly as the eosin staining bodies, and the nucleus stained more faintly blue than the nuclei of the surrounding liver cells. In most cases these bodies were without any nucleus; in others a nucleus was present. This always was at the periphery, and generally took the long irregular form of the nucleus of a wandering leucocyte. Polynuclear leucocytes were numerous in all the livers examined. In some cases there were well-defined groups of them in the capillaries and in the liver beam-work between, and as it seemed often in the red-stained bodies. In several specimens there were hemorrhages in the liver, large areas being occupied by red blood corpuscles between which the red bodies were often seen. This peculiar condition of the liver is possibly made more clear by staining the sections deeply with picro-carmine. In sections so treated these bodies stain an intense bright yellow with the picric acid. Concerning the nature of these bodies there can be little question. When first seen it was thought that they were probably some form of lower organisms, possibly amœbea, but a more extended study showed that this could not be so. Bodies in all respects similar to them were found in rapidly advancing cases of cirrhosis of the liver, in phosphorous poisoning, and in other cases of rapid fatty degeneration, but they are particularly found in cases of acute yellow atrophy of the liver. Areas were found in sections from this which were very similar to the advanced cases of yellow-fever liver. It must be considered that in yellow fever, along with the fatty degeneration, there is a necrosis of the liver cells which sometimes affects only portions of the cells; at others the entire cell. Almost every change leading up to the formation of these bodies could be seen. The exact relation of the fatty degeneration to the necrosis could not be determined. The necrotic masses were found both in intact liver cells and in those which had undergone fatty degeneration. In the latter cases it seemed probable that the necrosis preceded, or at least accompanied, the degeneration. If it only represented a necrosis of the small remnant of cell protoplasm between the fat drops it is difficult to see how so large a body could be formed from

this. When the necrotic masses were found in the liver cells they were nearly always at the periphery of the cell next to the capillary.

Although these necrotic masses were generally found in the beam-work of liver cells, careful examination showed them frequently to be in the hepatic veins and in the capillaries. It would have been interesting to have examined sections of the lungs to determine what part they might play in the formation of emboli.

The other changes of the liver consisted in infiltration of round granulation cells around the portal vessels. The fatty degeneration and the necrosis was much less marked in this place; even in the most advanced cases a certain number of normal liver cells were always found here. Some of the liver cells, particularly around the portal spaces and adjoining the portions most degenerated, contained several nuclei, generally oval, and less vesicular in appearance than the normal nucleus. Rows of smaller cells with similar nuclei were also found, and appeared to indicate an attempt at regeneration. A few of the specimens from autopsies very shortly after death and which were at once placed in alcohol showed rather ill-defined nuclear figures.

In the kidneys the changes were generally those indicating an intense parenchymatous degeneration. They were most marked in the tubules of the labyrinth. The glomeruli in the most cases were affected. The capsular space was dilated and filled with a granular exudation coagulated by the alcohol, and frequently in this, round hyaline masses were found. The epithelial cells of the convoluted tubules were very much swollen and the tubules often dilated. The cells often contained larger and smaller fat drops shown by the clear spaces remaining after this was dissolved out by the alcohol, but the principal change was a hyaline degeneration of the cells. The cells contained an immense number of clear hyaline granules which stained more brightly with eosin. In many cases there appeared to be a well-defined margin to the cells, and over this what appeared to be a row of cilia. Examination with high-power objectives showed this to be composed of oblong granules of the same hyaline material as that within the cells, and apparently represented an exudation. In the dilated tubules there were large and smaller, generally round, masses of similar hyaline material.

The most peculiar change in the kidneys in some cases was the presence of masses of colloid material and crystals in the tubules. This colloid material was found in the loops of Henle and in the collecting tubules. It was sometimes in the form of round masses and sometimes in irregular clumps, apparently formed from aggregation of the round masses. These were sometimes united to each other to form long chains. When these bodies are seen singly the center appears depressed and sometimes stained more deeply than the periphery. They stained with nearly all of the nuclear stains, but particularly with Bismarck brown and hæmatoxylin. They were highly refractive, and either perfectly hyaline or composed of numerous laminæ, like starch granules. They were generally found in the lumen of the tubules, but in one case there was a large aggregation between the epithelium and the wall of the tube. The crystalline masses were found in every case examined, and differed in all respects from the material just described. They were of a yellowish or yellowish-green color, with sharp edges, very highly refractive, and with numerous radiating lines and fractures running from the periphery to the center. They did not stain with any of the reagents used. In addition to these bodies, in some cases the tubules contained beaded strings of a substance evidently derived from the blood, and which stained intensely with both eosin and picric acid. Casts both epithelial and hyaline were found, the latter apparently composed of aggregations of the same hyaline material which was found in the epithelium. In some of the tubules considerable numbers of leucocytes were found, but in general it was remarkable, in view of the extent of the parenchymatous degeneration, how little cellular infiltration there was.

The changes in both the liver and kidney appear to be due to a general toxemia rather than to the local presence of infectious agents. They are diffuse, affecting the whole of the organs, and not small areas.

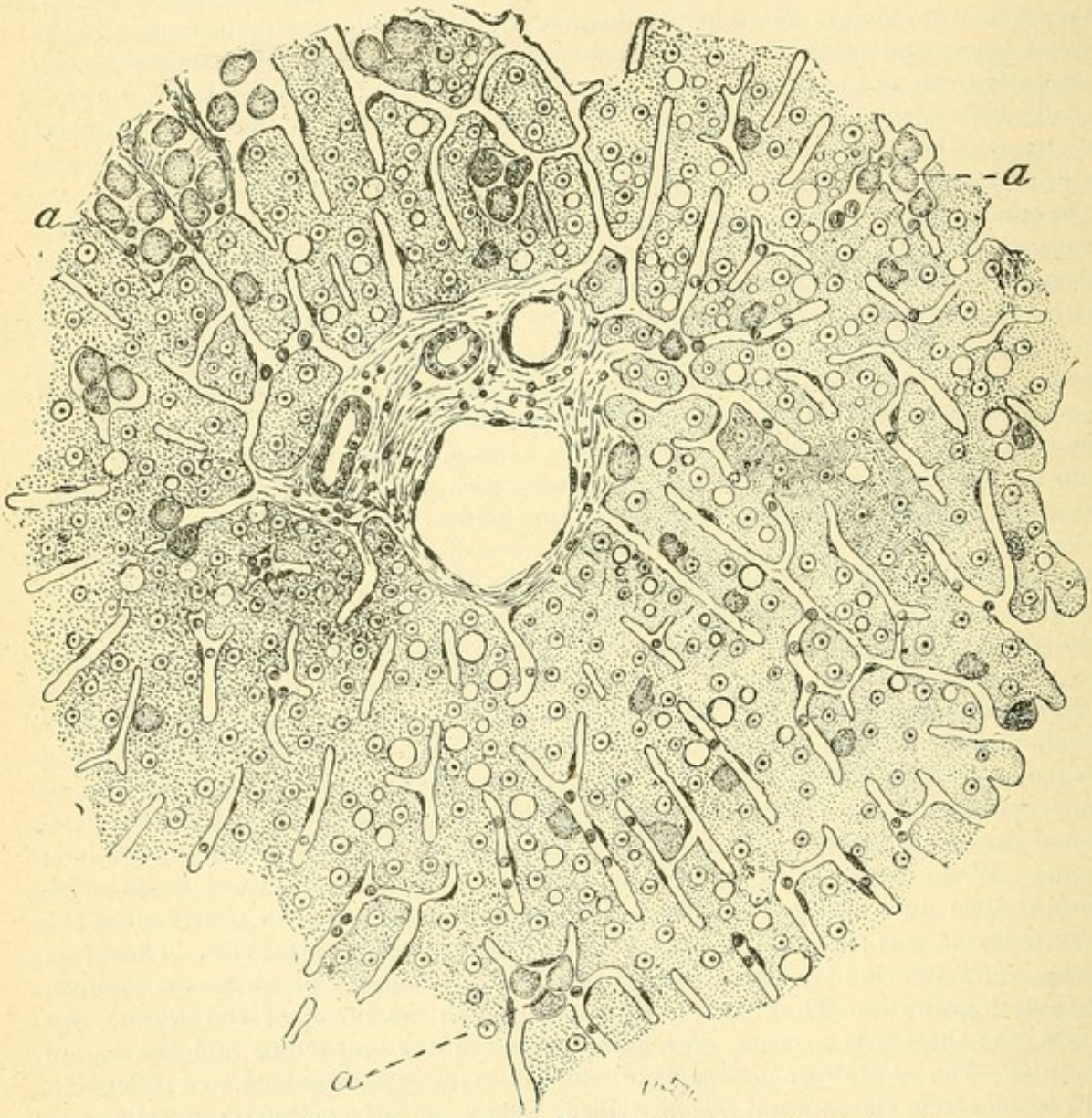
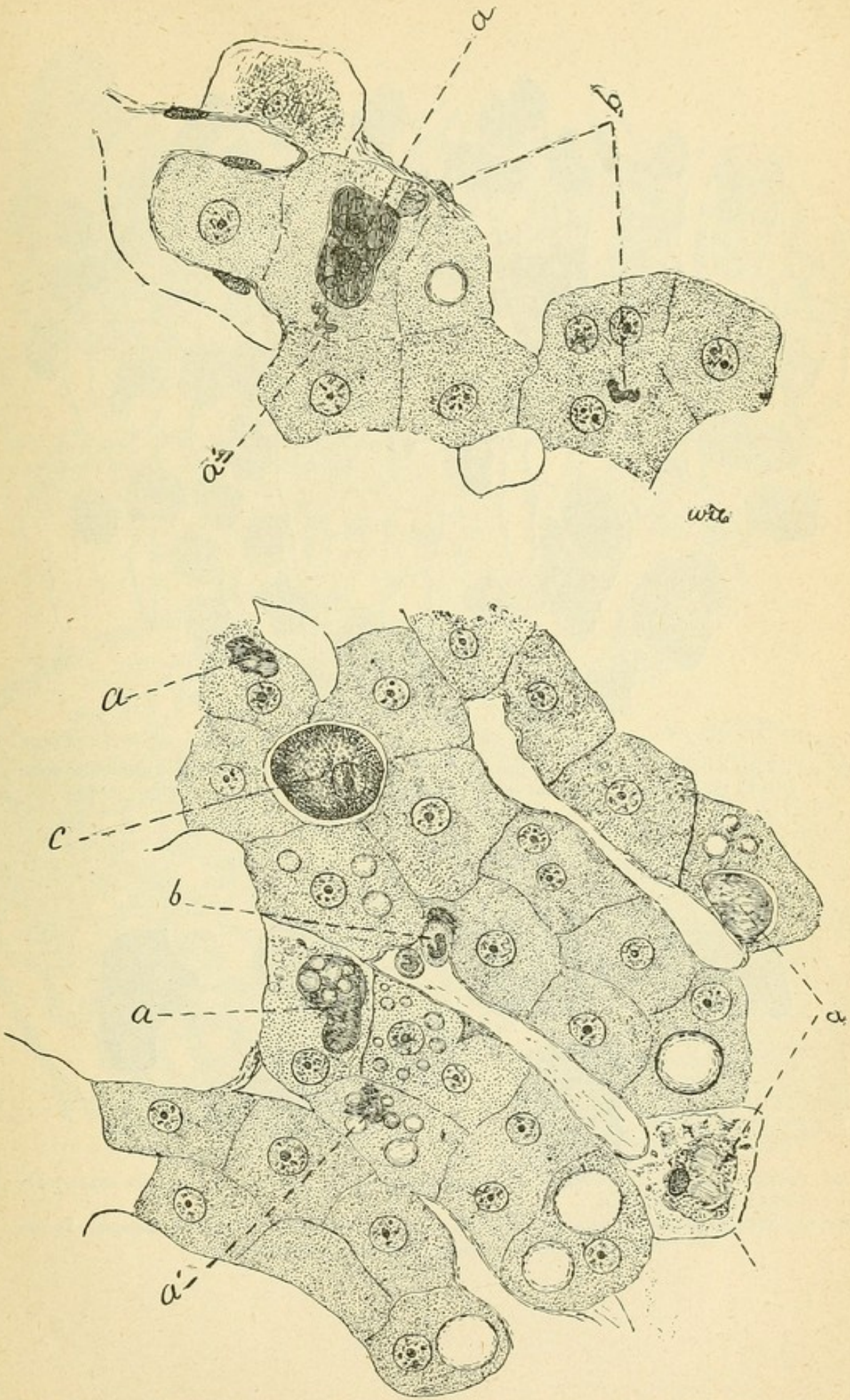


FIG. 10.—Section of liver but little affected. *a*, necrotic masses. × 150.



FIGS. 11 and 12.—From same liver. *a*, necrotic masses; *a*, small necroses in liver cells, not so sharply differentiated as the larger masses. *b*, leucocytes; *c*, liver cell, in beginning necrosis. × 400.

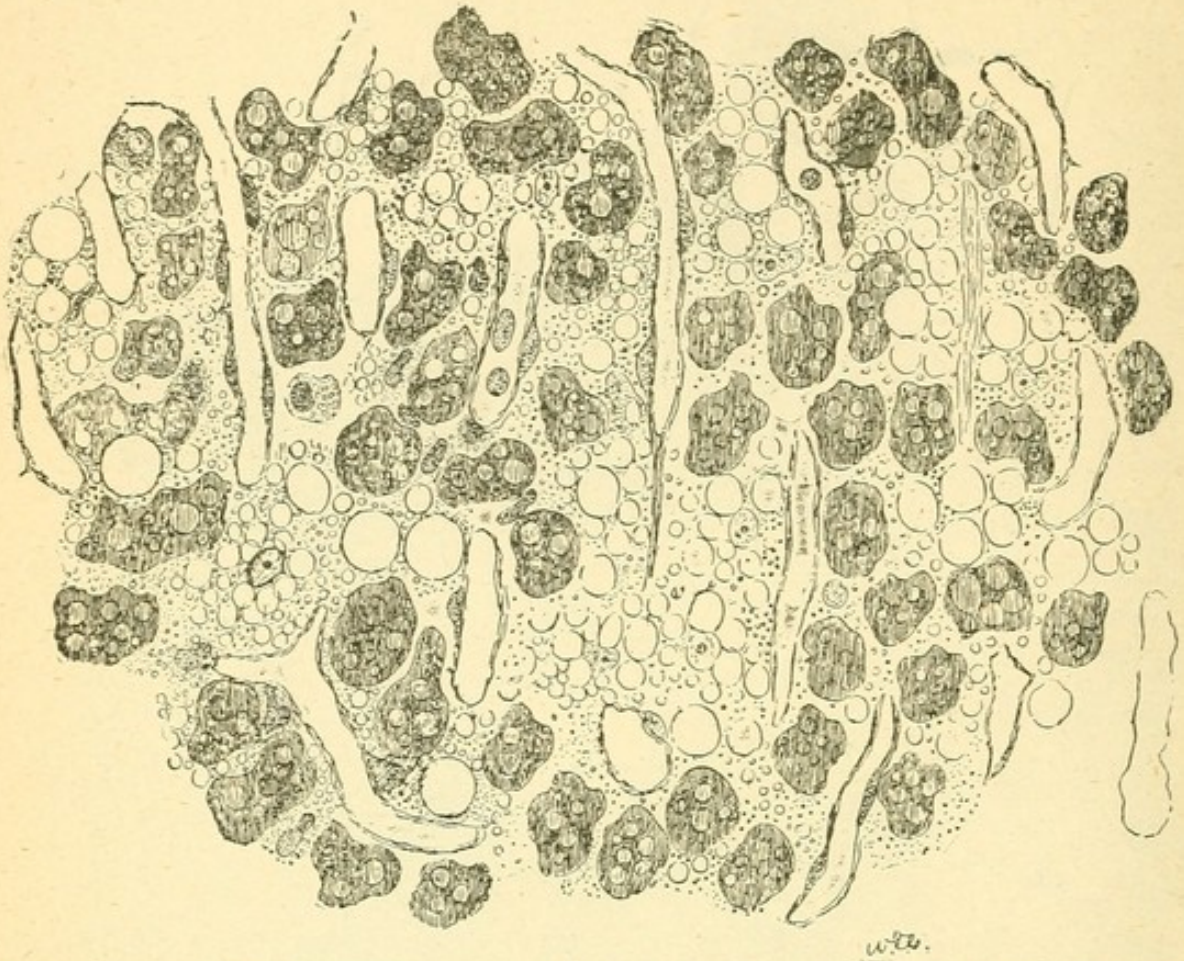


FIG. 13.—From a liver in an advanced stage of degeneration. The spaces between capillaries occupied by the necrotic masses; fat and débris of liver cells with a few leucocytes. × 300.

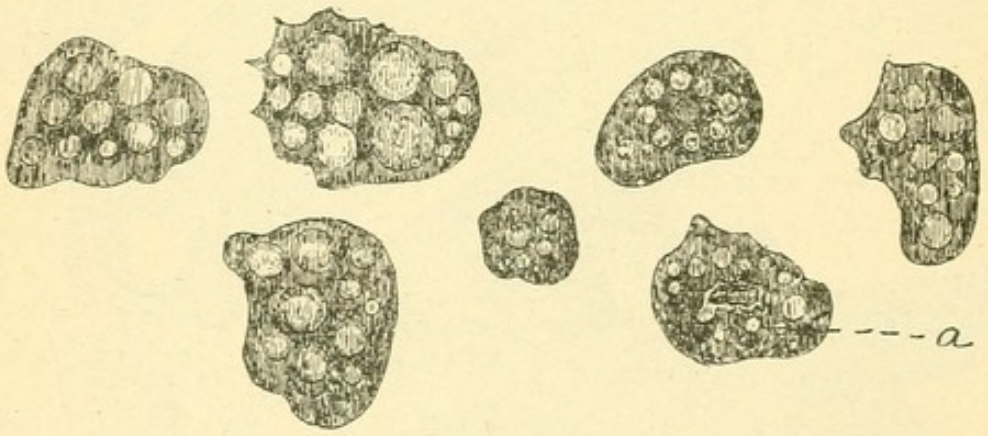


FIG. 14.—From same liver; necrotic masses with higher power, Leitz one-twelfth immersion. In one of the bodies *a*, a vacuole, inclosing a small body.

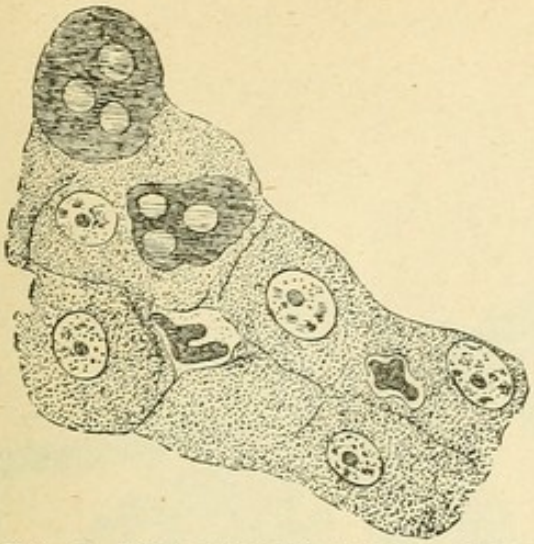


FIG. 15.—Liver cells, with necrotic masses, and small masses between and in the liver cells.

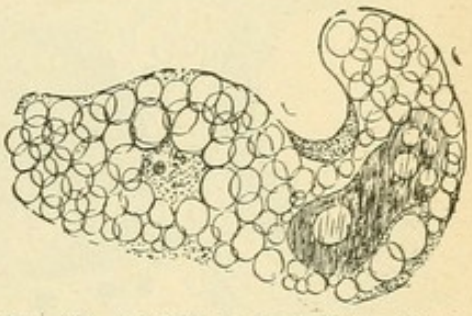


FIG. 16.—Necrotic mass in fatty, degenerated liver cells.

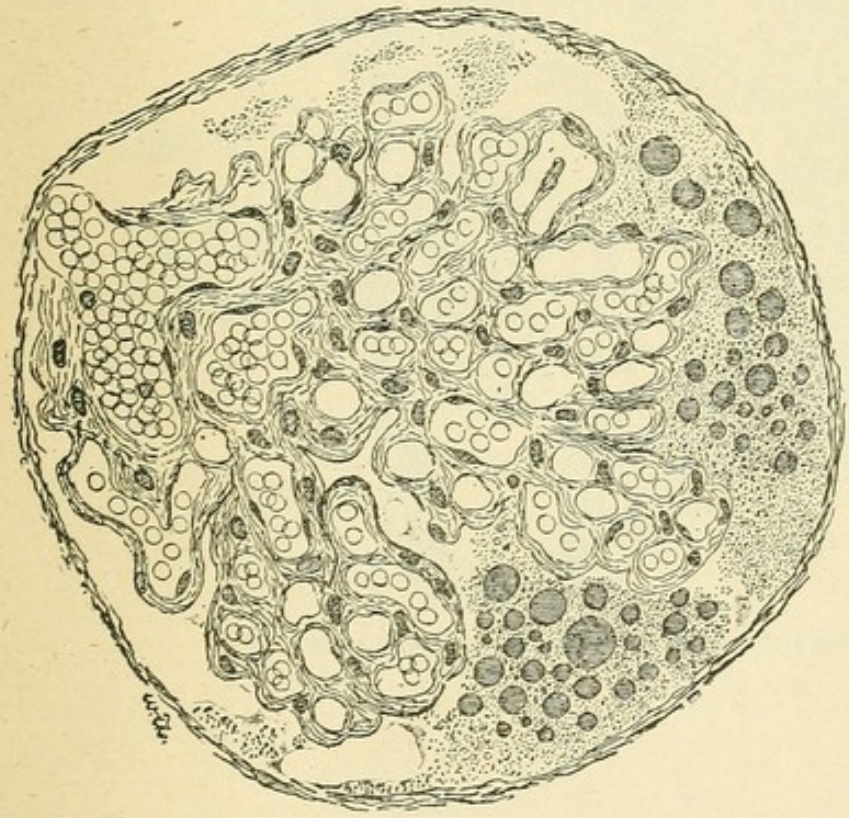


FIG. 17.—Glomerulus with granular and hyaline material in capsular space.

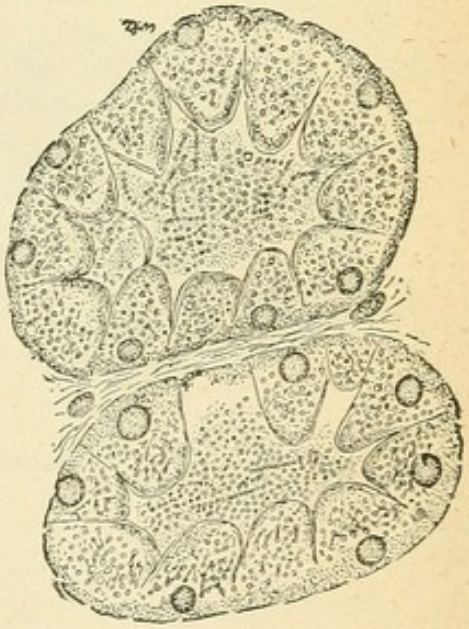


FIG. 18.—Section of kidney, with hyaline degeneration of epithelium and hyaline and granular material in the lumen of tubules.

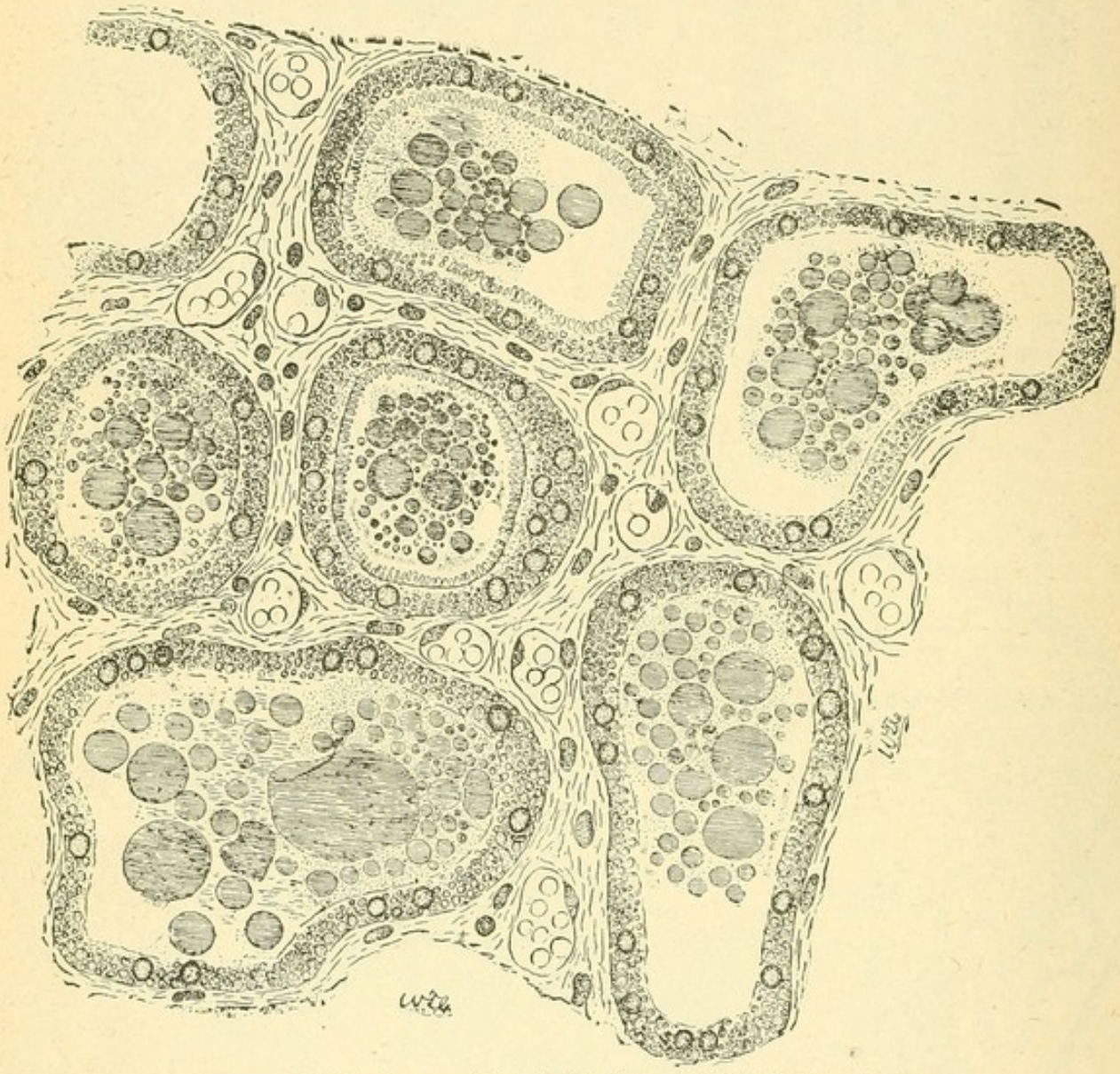


FIG. 19.—Hyaline degeneration of epithelium; Muller's fluid specimen.

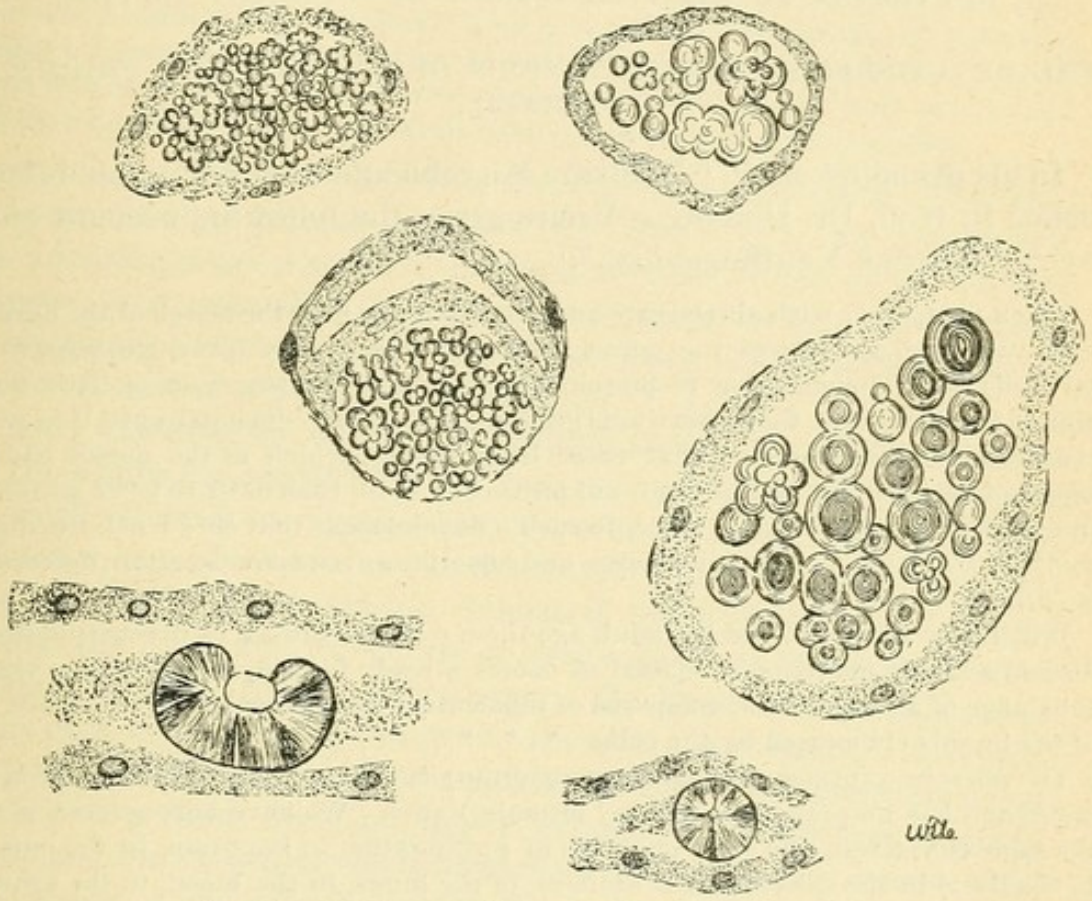


FIG. 20.—Tubules containing colloid material and crystals.

VII.—DESCRIPTION OF MICROÖRGANISMS WHICH HAVE BEEN CLAIMED TO BE THE CAUSE OF YELLOW FEVER.

THE CRYPTOCOCCUS XANTHOGENICUS OF DR. DOMINGOS FREIRE, OF BRAZIL.

In his principal work, "Doctrine Microbienne de la fievre jaune," published in 1885, Dr. Domingos Freire gives the following account of his "Cryptococcus Xanthogenicus":

When we follow, with all the care and attention possible, the march of the development which characterizes the germs which produce yellow fever, we acquire the certainty that, commencing to present themselves under the form of little points almost imperceptible, they afterwards gradually increase in diameter until they attain considerable dimensions, so that these little beings, which at the outset had the aspect of very little grains of sand, not measuring more than 0.001 to 0.002 millimetre in diameter, arrive, little by little, to such a development that they reach the dimensions of 0.005, 0.007, or 0.008 millimetre, and sometimes even more in certain determined conditions.

When they have attained the adult age these cells are broken at diverse points and discharge their contents, composed of spores already formed, mixed with a viscous substance of a yellow color, composed of pigmentary and protoplasmic substance, and of the liquids elaborated by the cells. * * *

The microbe xanthogenicus is a cosmopolitan; it does not select its domicile in any organ and has no preference for any organic liquid. We have encountered it with the same characters, the same opulence of proliferation in the brain, in the muscles, in the liver, in the spleen, in the kidneys, in the lungs, in the blood, in the urine, in the bile, in the vomit, and even in the cephalo-rachidian fluid. However, it is necessary to establish a well-drawn distinction as to the blood. The blood of the general circulation shows itself much less charged with the microbes than the blood of the capillaries. Thus, if I could admit any preference on the part of the microbe xanthogenicus, I would say that it pleases itself better in the blood of the capillaries, in the blood which bathes immediately the anatomical elements. * * *

The occasion seems to us a favorable one in order to call attention to some indispensable precautions when the microbes of yellow fever are to be sought in organic solids and liquids. While it is extremely easy to perceive the presence of the microbes of yellow fever in the urine and bile, for example, by placing a drop of these liquids upon a glass slide, covering it with a thin glass cover, and examining it with a power of 450 to 740, or 780 diameters, this proceeding can not be employed when the blood is to be examined. If we proceed in this manner the globules will hide nearly all the microbes and the observer will wrongly conclude that they are very rare in this organic liquid. Not only does the form of the microbe offer a certain resemblance to that of the red corpuscles, but these latter in adhering together envelop the microbian cells, and, on the other hand, cast upon the cells a jet of light, which makes them disappear from the field of the microscope. But if we dilute a little drop of blood in a pure solution of sulphate of soda and place it under the objective, the microbes become visible and will appear in considerable quantity.

It is likewise necessary to make a preparation previously for the examination of the cerebral mass and of the muscles. They should be triturated in a sterilized mortar and mixed afterward with distilled water entirely deprived of organisms, filtered through fine linen which has been passed rapidly through the flame of an alcohol lamp, and afterwards a drop of the filtered liquid should be placed upon a glass slide. If we withdraw a little piece of brain or of muscular fiber, even triturated, we will not perceive anything abnormal under the microscope unless it be the anatomical elements more or less deformed by trituration.

It is not the same for the liver. It suffices to withdraw a bit of this organ and to crush it between two glass slides; upon observing it under the microscope we perceive at once a multitude of microbes. This is because in the muscles the microbes are lodged between the fibrillæ and in the substance which surrounds them, and in the brain they are found in the interior of the nerve-cells, which must first be destroyed by trituration in order that their parasitic hosts may become visible.

I would say in the first place that the description above given of the cryptococcus xanthogenicus does not correspond with the characters of the microörganism which Dr. Freire presented to me as his yellow fever germ; and, secondly, that no such organism as he has described or as was present in the cultures which he gave me, is to be found in the blood or tissues of yellow-fever patients.

The only explanation of the remarkable versatility as to the form which Dr. Freire has ascribed to his "cryptococcus" on different pages of the work referred to, which I can conceive of is that offered by Dr. Araujo Goes, one of his medical confrères and critics in Rio de Janeiro, viz, that Dr. Freire has mistaken deformed blood corpuscles, fat globules from the liver, and the débris of tissue elements in his trituration of muscle, brain, etc., for microörganisms. Dr. Freire frequently speaks of his cryptococcus as being endowed with active movement. It is well known to microscopists that minute particles, organic or inorganic, when suspended in a fluid, undergo, under certain circumstances, a rapid vibratory motion, known as the brownian or molecular movement. The microörganism, a micrococcus, which Dr. Freire presented to me as his yellow-fever germ, like other similar organisms, presents these molecular movements when suspended in a fluid, but it has no proper vital movements, such as are manifested by many of the bacilli. The fat drops from crushed liver tissue, or the débris of muscle fibrillæ, present these molecular movements, and in form and appearance present some resemblance to micrococci. They are easily distinguished, however, by chemical tests and staining agents. I may remark here that prior to his visit to Paris in 1887, Dr. Freire seems not to have made use of the method of staining with aniline dyes. In an address, made while in Paris, he defends himself from the charge which some one seems to have made, that he had neglected this means of recognizing microörganisms, in the following language:

We know that in order to color a microbe it is necessary, first, to kill it and then to wash the little microscopic cadaver by means of reagents possessing the power of dissolving all matters foreign to its skeleton. At the outset I applied myself to the study of the yellow-fever microbe in a fresh state. I fed it with the best food, *les*

meilleurs engrais, for the purpose of witnessing the different phases of its evolution from its birth to its death.

Nevertheless, in fault of other accusations, some authors have reproached me with not having colored my microbe. Alas, what a miserable objection! Is it necessary in order to affirm the existence of a microbe, which swarms by millions in the urine, in the bile, in the blood, in the tissue, etc.; is it necessary to mask them, to disguise them under a costume of carnival, in order to please certain microscopists? M. Pasteur has never colored his microbes; and, nevertheless, everyone admits the existence of the bacillus of charbon, of the corpuscles of pébrine, of the micrococcus of fowl cholera, etc.

* * * Do not think, gentlemen, that I fear the application of coloring processes to the search for the microbe of yellow fever. Far from it. In order to show you that the criticism which I have just made is not due to prejudice, I will say to you that such processes have recently been employed upon the yellow-fever microbe with complete success.

The method of cultivating in solid media and of isolating microörganisms by means of plate cultures or Esmarch tubes, seems also to have been unknown to Freire prior to his visit to Paris. All of the cultures left in his laboratory at the time of my arrival were in liquid media and preserved in Pasteur flasks. He brought from Paris, however, a number of cultures in agar-agar, and among them one which he presented to me as a pure culture of his yellow fever microbe. This was a micrococcus, which multiplies, as do other microörganisms of the same class, by binary division and not by the growth of the separate cells to comparatively large dimensions, and the formation of endogenious spores, which are released by rupture of the cell wall of the mother cell, as described by Dr. Freire. This mode of multiplication is not known among the bacteria, and does not occur in the micrococcus which Dr. Freire placed in my hands as his yellow fever microbe, and which I have had in cultivation continuously since my visit to Brazil in 1887. This is a simple staphylococcus which multiplies by binary division, and upon the surface of nutrient agar forms a milk-white mass. I have never seen it produce either the yellow or the black pigment which Dr. Freire ascribes to it. In an address made by him in Paris, in April, 1887, he repeats the statement previously made in his principal work, in the following language:

Each adult cell is ruptured in one or several points and allows to escape its contents composed of germs which are to perpetuate the species, and two pigments, one yellow, destined to infiltrate the tissues and produce the icteric color which has given name to the malady, the other black, insoluble, and destined to be carried along by the circulatory current, producing either capillary obstructions or blood stasis in the parenchyma of the organs.

This account is entirely fanciful. Not only does Dr. Freire's micrococcus not produce such pigment as he describes, but in yellow fever no black pigment granules are found in the capillaries or the parenchyma of the organs, as is the case in malarial fevers. Moreover the pigment which gives a black color to the vomited matters, and to the contents of the intestine in most fatal cases, is fully accounted for and does not

result from the presence of Dr. Freire's crpytococcus or of any other microörganism. It is blood pigment changed by the acid secretions of the stomach and present in that viscus as a result of passive hemorrhage from the mucous membrane. This I have verified very many times by microscopical examinations which have shown the granular brown pigment, which gives color to the "black vomit," to be closely associated with little masses of decolorized blood corpuscles; and it has been demonstrated by a spectroscopic examination made at my request by Dr. George Kemp, of the Hoagland laboratory. Dr. Freire's micrococcus liquefies gelatine quite slowly, forming a cup shaped cavity, at the bottom of which the cocci accumulate, forming a white deposit, as shown in Fig. 5, Pl. III. The morphology is shown by my photomicrograph from a preparation stained with fuchsin, Fig. 1, Pl. III.

That this micrococcus bears no relation to the etiology of yellow fever is fully proved by my extended culture experiments in Havana during the summers of 1888 and 1889. In the entire series of autopsies I have made cultures from the liver, and in a considerable number from blood obtained directly from the heart, and I have not obtained this micrococcus of Freire in a single instance, although the culture medium commonly employed—flesh peptone-gelatine—is a very favorable one for the growth of this coccus. Nor has it been found in the extended series of sections which I have made from the liver and kidney preserved in alcohol from my Havana autopsies. In one case only (case 10, 1888) I have found micrococci in sections from the kidney, but as the micrococcus of Freire, in its form and dimensions, resembles many others, it is impossible to say that this is or is not the Freire coccus. The finding of micrococci in this case, however, does not invalidate the general result, which is that *micrococci are not found in the blood and tissues of yellow fever cadavers.*

The micrococcus of Freire is killed by 10 minutes' exposure to a temperature of 60° C. (140° F.). Its vitality is not destroyed by a freezing temperature. I have subjected it for an hour and a half to a temperature of 15° C. below zero (5° F.) in a freezing mixture of salt and ice, and found that it grew as usual when planted in flesh-peptone-gelatine, and kept at a temperature of 26° C. A stick culture in the same medium, placed in an exposed attic in the laboratory in Baltimore, from January 17 to 29, at a temperature ranging from 2° to 10° C., not only was not killed, but showed decided growth. In short it is a hardy micrococcus, which grows at comparatively low temperatures and preserves its vitality in sealed tubes for several months.

THE YELLOW-FEVER "GERM" OF DR. CARMONA Y VALLE, OF MEXICO.

Dr. Manuel Carmona y Valle has given an account of his researches and supposed discovery in his memoir, entitled, "Leçons sur l'étiologie et la prophylaxie de la fièvre jaune," Mexico, 1885.

I am indebted to Dr. Carmona for a copy of this work, which he presented to me at the time of my visit to Mexico. The following inscription, signed by him, is upon the title-page, and is a frank acknowledgment that the author has modified his views very considerably with reference to his earlier observations:

A. M. le Dr. STERNBERG:

Quoiqu'il soit nécessaire de modifier toute la partie relative à la morphologie et aux cultures du microganisme.

Mexico, le 26 Septembre, 1887.

DR. CARMONA Y VALLE.

At the time of my visit to his laboratory (September, 1887) Dr. Carmona was inclined to attach some importance to a bacillus which he had obtained in his cultures from yellow-fever urine sent to him from Vera Cruz. This bacillus is shown in my photomicrograph, Fig. 4, Pl. III, which is from a slide mounted by Dr. Angel Gaviño Yglesias in Dr. Carmona's laboratory. Associated with the large bacillus, shown in the photomicrograph, there is another slender bacillus in smaller numbers, which is seen upon looking over the slide. This large bacillus was also present in a culture from yellow-fever urine which Dr. Carmona presented to me, and which, by the use of Esmarch roll tubes, I found to contain several other bacilli and a micrococcus. The large bacillus is of the "subtilis" group. It liquefies gelatine quite rapidly, and forms large oval spores.

Not having obtained it in my cultures from the blood and tissues of yellow-fever cadavers, or from urine drawn through the walls of the bladder in a considerable number of cases, I must regard its presence in Dr. Carmona's cultures from urine collected for him in Vera Cruz as entirely accidental, and without significance so far as the etiology of yellow fever is concerned.

THE YELLOW-FEVER GERM OF DR. CARLOS FINLAY, OF HAVANA.

(*Micrococcus tetragenus versatilis*, Sternberg.)

Dr. Carlos Finlay having, in his earlier researches, observed micrococci in groups of four in cultures obtained from mosquitoes which he had allowed to fill with blood from yellow fever patients, inferred that the micrococci came from the blood of the sick, and that the grouping in fours was a character by which he could distinguish this microorganism, which he named *Micrococcus tetragenus febris flavæ*, upon the supposition that it was concerned in the etiology of yellow fever.

During the winter of 1887–'88 Dr. Finlay sent me a number of his "mosquito cultures," which I found to contain a variety of microorganisms. Among these a large micrococcus grouped in tetrads was most conspicuous. This I isolated and studied in pure cultures, and have since named *Micrococcus tetragenus versatilis*, a name which Dr. Finlay has accepted.

The characteristic mode of grouping is shown in Fig. 3, Pl. III. Some bacteriologists would perhaps be disposed to place it among the Sarcinæ, but I have never observed any evidence of division in a third plane, forming packets of eight or more elements, such as are characteristic of this genus. I have called it "versatilis" because it is very versatile both in the grouping of the elements and in their dimensions. In the same culture very wide differences in size are observed, and at different times and in different media these variations are very noticeable. The grouping also varies greatly; sometimes the greater number of the elements are arranged in tetrads, or in pairs in which the large oval elements are upon the point of dividing transversely to the line by which the binary division of a single element is marked. But often there are irregular groups of three or more elements, or there may be a chain of tetrads which remain attached one to the other.

In agar-stick-cultures a rather thick and viscid yellow mass is formed on the surface, about the point of puncture; and in the course of a week or ten days, at a temperature of 20° to 25° C., this extends over the entire surface. The color varies from cream yellow to lemon yellow. The growth upon potato is similar to that upon agar. In stick cultures in flesh-peptone-gelatin the gelatin is liquefied rather slowly near the surface, forming a deep cup-shaped cavity, as shown in Fig. 7, Pl. III. Colonies in gelatin roll-tubes are at first pale yellow and later lemon yellow in color; they are opaque and spherical, and do not usually cause liquefaction of the gelatin for several days. The microörganism has no proper movements and is aërobic, no growth occurring in an atmosphere of hydrogen. It is not pathogenic for rabbits or guinea pigs.

I have occasionally obtained a few colonies of this micrococcus in my cultures from the contents of the stomach and intestine of yellow-fever cadavers, and in one case (case 8, 1888) I obtained it from the liver kept in an antiseptic wrapping for 48 hours. In this case it was associated with *staphylococcus pyogenes aureus*. There is no reason to believe that it has anything to do with the etiology of yellow fever. My researches show that it is a very common organism upon the surface of the body of patients in the hospitals of Vera Cruz and of Havana, and quite as common in cases not having yellow fever as in those sick with this disease. In Brazil, in 1887, my friend Dr. Goes obtained it in a culture from blood drawn from the finger of a yellow-fever case in the small-pox hospital. My inference then was that its presence was accidental, and due to contamination of the drop of blood while collecting it; and I believe this to have been the case when it has been present in Dr. Finlay's cultures from blister serum. In a case of brain disease, and in a case of skin disease, in which Drs. Finlay and Delgado applied blisters and collected serum by their usual method, I obtained this micrococcus in Esmarch roll-tubes to which this serum had been added. These cases had not been associated in any way with yellow-fever patients,

and the blister serum was collected at my suggestion as a control experiment, inasmuch as Drs. Finlay and Delgado had obtained this micrococcus in their "mosquito cultures," and in blister serum from yellow-fever cases. Its absence in cultures from the blood in a large series of cases fully supports the inference that its occasional presence in blood drawn from the finger, or in blister serum, is due to accidental contamination from the surface of the body or from the atmosphere.

Dr. Finlay himself, when I left Havana last year, had about given up the idea that this tetragenus is the cause of yellow fever. This is shown by the following letter from him:

HAVANA, *August* 29, 1889.

MY DEAR DOCTOR: I send you a copy of the résumé of our investigations during the year, May, 1888–'89, which Dr. Delgado and myself presented at the beginning of the year. You will see that we did not claim to have *demonstrated* that our "tetragenus" was the actual germ of yellow fever, but merely that in our recent investigations, carried out with methods which we deemed to be reliable, we had again found the same microörganism in yellow fever finger blood and in blister serum, and also in cadaveric products of two yellow fever autopsies. We likewise expressed the hope that you would undertake comparative experiments in order to determine, first, whether it was a fact that by the culture methods which we had described our tetragenus could be demonstrated in most of the products collected during life from yellow fever patients, and, second, whether that microörganism is exclusively found in such patients. I am aware that the results of three samples of yellow fever blister serum and seven samples of blister serum from acclimated subjects have given a negative answer on the second point. Yet I can not wholly divest myself of the suspicion that the greater frequency with which we have found the tetragenus in our yellow fever cultures (from material collected during life) may have some significance, even admitting, as I do, that before any etiological importance can be claimed for it quite a number of serious objections would have to be encountered besides showing that it is not to be found in localities where yellow fever is unknown.

I am, my dear doctor, yours, very faithfully,

CARLOS FINLAY.

Dr. G. M. STERNBERG, U. S. Army,
Havana.

Finally, I may say that this "tetragenus" is comparatively large, and conspicuous by reason of its grouping in tetrads, and that it is promptly stained by the aniline colors. It should therefore be found in my sections of the various organs if present in the blood of yellow fever patients. It has not been found in the numerous sections made from forty cases in which I have made autopsies in Havana, unless possibly the cocci found in a single case in sections from the kidney (case 10, 1883) are identical with Dr. Finlay's tetragenus. As remarked in the account of Dr. Freire's coccus, such identity can not be established by microscopic examination alone, and the question, which is of little importance in view of the facts stated, must remain unanswered.

But one thing is very evident, and that is that the researches of Dr. Finlay give no support to the claims of Freire, inasmuch as the micrococcus which has especialiy engaged his attention is entirely different from that of the Brazilian investigator.

THE BACILLUS OF DR. PAUL GIBIER.

(*Bacillus lepina lethalis*, Sternberg.)

Dr. Paul Gibier, a French bacteriologist, went to Cuba in the autumn of 1887 in the expectation of finding the yellow fever microbe of Dr. Freire, who had spent some time in his laboratory in Paris. This is shown, as well as his failure to realize this expectation, in the following communication made by him to the French Academy of Sciences:

HAVANA, *January* 22, 1888.

At the commencement of the year 1887 Dr. Domingos Freire, professor in the faculty of medicine of Rio de Janeiro, came to Paris in order to present to the scientific public his studies upon yellow fever. M. Freire was presented to me by Dr. Rebourgeon, who had studied this malady with him in Brazil. The laboratory of comparative pathology of the museum was opened to these savants, who resumed the experiments, the results of which had previously been published by M. Freire. I was requested by Dr. Freire to examine the cultures which he had brought with him, and to treat them by the new bacteriological methods, which had not yet been applied in his researches. After these investigations, made in common, M. Freire had the kindness to associate me in a communication made in his own name and that of Rebourgeon to the Academy of Sciences during the month of March, 1887.

Since, and as a result of this communication, I received from the minister of public instruction the mission to go and "study yellow fever in the countries where it prevails habitually, and the prophylactic measures which may be opposed to this malady."

Dr. Gibier arrived in Havana in November, 1887, and proceeded to make bacteriological researches by approved methods, the results of which he announces as follows in the communication from which we have quoted:

Results obtained.—I am obliged to confess here, however much it may cost me, that my results contradict in an absolute manner the facts advanced by M. Domingos Freire, from whom I have the regret, as well as the duty, to separate myself.

The blood.—In a great number of preparations, fresh or colored, it has been impossible for me to verify the presence of microörganisms. The cultures repeated a great number of times remained sterile. * * *

The numerous sections which I have made of the different viscera have also failed to show me the presence of microbes.

Having convinced himself that neither the micrococcus of Freire, nor any other microörganism was present in the blood of yellow fever patients, Dr. Gibier turned his attention to the microörganisms present in the alimentary canal, and isolated from the contents of the intestine of one or more cases a liquefying bacillus to which he was inclined to attach especial importance.

Dr. Gibier kindly placed in my hands a culture of this bacillus upon my arrival in Havana in the spring of 1888, and I have had it in constant cultivation since that time, and have made numerous inoculations into rabbits and guinea pigs which show that it is pathogenic for these animals.

But my extended researches give no support to the supposition that it is concerned in the etiology of yellow fever. In a large majority of

my cases it has not been present in cultures made from the contents of the stomach and intestine obtained post-mortem, or from the alvine discharges obtained during the life of the patient. I obtained it in comparatively small numbers in three out of ten cases in which I made autopsies in Havana in 1888.

Admitting to myself the possibility that the specific germ of the disease might be absent, or only occasionally present, in the contents of the intestine obtained post-mortem, although present at the outset of an attack, I devoted myself especially to a search for this bacillus in the feces of yellow fever patients during my stay in Decatur, Alabama, in the autumn of 1888. The result of this research was to show that liquefying bacilli are not numerous in the alvine discharges of the sick, and that the bacillus of Gibier was only present in a limited number of cases and in comparatively small numbers. Another liquefying baccillus, *o*, was found more frequently, but not with sufficient constancy to give support to the belief that it bears an etiological relation to the disease.

Again, in my extended researches in Havana during the summer of 1889 I have only encountered this bacillus in my cultures from the stomach and intestine in a limited number of cases.

I give below some of my notes with reference to the presence of liquefying bacilli:

Autopsy No. 14 (*No.* 1 *of* 1889).—"*B coli commune* in anaërobic and aërobic culture from stomach and intestine, no liquefying colonies."

Autopsy No. 15.—"No liquefying colonies in cultures from intestine. A few liquefying colonies in Esmarch roll tube No. 1 from stomach. Liqueying bacillus with large oval end spore in anaërobic Esmarch tube from intestine."

Autopsy No. 16.—"Liquefying bacillus (Bacillus *o* Decatur) in gelatine Esmarch tubes from stomach and intestine."

Autopsy No. 17.—"Cultures from intestine, bacillus *a* and a single liquefying colony, not Gibier's bacillus."

Autopsy No. 18.—No remarks made with reference to liquefying bacilli, which indicates their absence.

Autopsy No. 19.—"No liquefying colonies from stomach or intestine at end of 24 hours."

Autopsy No. 20.—"Bacillus *g*," (Gibier's bacillus). "Obtained from intestine. Esmarch roll rubes No. 1 and No. 2 both liquefied at end of 24 hours."

Autopsy No. 21.—"Gelatine Esmarch No. 1 from intestine liquefied in 48 hours; bacillus *ee*" (quite different from Gibier's).

Autopsies Nos. 22 and 23.—No notes, indicating absence of liquefying bacilli.

Autopsy No. 24.—"Gelatine Esmarch roll tube No. 1 liquefied in 24 hours; No. 2 on third day; bacillus *g*."

In this case Gibier's bacillus was present, but in comparatively small numbers, because Esmarch tube No. 3 of the series did not contain any liquefying colonies.

Autopsy No. 25.—"No liquefaction of gelatine Esmarch tubes from intestine."

Autopsy No. 26.—"Doubtful case, diagnosis not supported by the pathological appearances; excluded."

Autopsy No. 27.—"Liquefying bacillus in gelatine Esmarch tube No. 1 from intestine." What bacillus was not determined.

Autopsy No. 28.—"No liquefying colonies in gelatine Esmarch tubes from intestine."

Autopsy No. 29.—"Gelatine Esmarch tube No. 1 liquefied in 25 hours; single colony in No. 2; No. 3 contains nonliquefying colonies only. The liquefying bacillus present is bacillus *g*." (Gibier).

Autopsy No. 30.—"No liquefaction of Esmarch tubes from intestine in 48 hours."

Autopsy No. 31.—"No liquefaction of gelatine Esmarch tubes from intestine at end of 48 hours."

Autopsy No. 32.—"No liquefaction of Esmarch tubes from intestine in 48 hours."

Autopsy No. 33.—"Esmarch tube No. 1 from intestine contains liquefying colonies at end of 48 hours; bacillus *g*; none in tubes Nos. 2 and 3, which contain the colon bacillus."

These notes from twenty successive cases will suffice to show what has already been stated, viz: that this bacillus is only exceptionally present, and when present is in comparatively small numbers. We are therefore obliged to exclude it from consideration from an etiological point of view.

I may here say that in my cultures made directly from the pure culture which Dr. Gibier placed in my hands, and from the intestinal contents of cases in which I made the autopsy, I have never seen any formation of black pigment such as Dr. Gibier described in his first communication to the French Academy of Sciences with reference to the bacillus under consideration.

The morphology of Gibier's bacillus is shown in Figs. 1 and 2, Pl. IV.

It is an actively motile bacillus with round ends, which varies considerably in length, being sometimes short oval, and again, in the same culture, long oval; or it may grow out into a flexible filament of considerable length. In recent cultures the bacilli are often united in pairs, and are deeply stained by an aqueous solution of fuchsin, or methylene blue; in cultures which are several days old, or in recent cultures when the stained preparation is washed in alcohol, the ends of the rods are commonly more deeply stained than the central portion. My photomicrograph is from the surface of an agar culture 12 days old, and shows some of the bacilli of recent development deeply stained, while others in the same field are but faintly stained.

This bacillus liquefies gelatine, as shown in Figs. 5, 6, and 7, Pl. IV.

At the end of 24 hours, at a temperature of 20°, to 22° C., there is an abundant development along the line of puncture and commencing liquefaction at the surface. Later the liquefaction is funnel-shaped, and there is an opaque white central core along the line of puncture with liquefied gelatine around it. Liquefaction progresses most rapidly at the surface, and in the course of 3 or 4 days the upper portion of the gelatine, for the distance of half an inch or more, is completely liquefied, and an opaque white mass, composed of bacilli, rests upon the surface of the unliquefied portion.

In gelatine roll-tubes the young colonies upon the surface are transparent and resemble somewhat small fragments of broken glass (see Fig. 3, Pl. IV); later liquefaction occurs rapidly, and the colonies are as seen in Fig. 4, Pl. IV. Deep colonies in gelatine roll-tubes or at the

bottom of gelatine stick-cultures are spherical, translucent, and of a pale straw color.

Upon the surface of nutrient agar it grows rapidly, forming a rather thin, translucent, shining, white layer, which covers the entire surface at the end of 2 or 3 days at a temperature of 20° C. (See Fig. 3, Pl. XX.)

Upon potato the growth is rapid and thin, covering the entire surface, and is of a pale yellow color.

This bacillus grows at a comparatively low temperature, and its vitality is not destroyed by exposure for an hour and a half in a freezing mixture at 15° C. below zero (5° F.).

Decided growth occurred in a stick culture in gelatine exposed in Baltimore during the month of January in an attic room (January 7 to 29). During the 22 days of exposure the highest temperature, taken at 9 a. m. each day, was 11° C., and the lowest, 2° C. At a temperature of 16° to 20° C. development in a favorable culture medium is rapid.

There is no evidence that this bacillus forms spores; cultures are sterilized by exposure to a temperature of 60° C. for 10 minutes.

Coagulated blood serum is liquefied by this bacillus. It retains its vitality for a long time in old cultures, having grown freely when replanted at the end of a year from a hermetically sealed tube containing a pure culture in blood serum.

My experiments on animals are given below in tabular form:

Animal.	No.	Size.	Date.	Where injected.	Amount.	Result.
			1888.			
Rabbit.....	11	.....	May 28	Cavity of abdomen.	½ cc. bouillon culture.	Recovered.
Do.....	27	Very small..	Aug. 16	Subcutaneous.....	3 cc. bouillon culture.	Do.
Do.....	28	....do	Aug. 16	....do	6 cc. bouillon culture.	Found dead at end of 16 hours.
Do.....	29	....do	Aug. 16	Cavity of abdomen.	3 cc. bouillon culture.	Do.
Do.....	30	420 grams...	Aug. 20	Subcutaneous	....do	Died at end of 48 hours.
Do.....	31	425 grams...	Aug. 20	....do	10 cc. bouillon culture.	Died at end of 2 hours.
Do.....	32	420 grams...	Aug. 20	....do	6 cc. bouillon culture.	Found dead at end of 20 hours.
Do.....	35	400 grams...	Sept. 4	Cavity of abdomen.	2 cc. gelatin culture.	Do.
Do.....	46	Large.....	Sept. 11	....do	4 cc. gelatin culture.	Found dead at end of 4 hours.
Do.....	52	Small.....	Sept. 24	....do	3 cc. gelatin culture.	Died at end of 3 hours.
Do.....	53	....do	Sept. 24	....do	....do	Died at end of 4½ hours.
Do.....	55	Large.....	Sept. 27	....do	2½ cc. gelatin culture.	Recovered.
Do.....	56	Small.....	Sept. 27	....do	1½ cc. gelatin culture.	Found dead at end of 20 hours.

Animal.	No.	Size.	Date.	Where injected.	Amount.	Result.
			1888.			
Rabbit.....	57	Small.....	Nov. 5	Subcutaneous	2 cc. gelatin culture.	Recovered.
Do.....	61	555 grams...	Dec. 5	....do	....do	Found dead at end of 20 hours.
Do.....	63	720 grams...	Dec. 12	....do	3 cc. gelatin culture.	Died at end of 24 hours.
Do.....	65	750 grams...	Dec. 15	Cavity of abdomen.	1 cc. gelatin culture.	Found dead at end of 20 hours.
Do.....	68	874 grams...	Dec. 20	....do	½ cc. gelatin culture.	Found dead next morning.
Do.....	69	720 grams...	Dec. 18	Small intestine	½ cc. gelatin culture.	Result negative.
Do.....	70	.....	Dec. 18	....do	½ cc. gelatin culture.	Do.
			1889.			
Do.....	71	1,350 grams.	Jan. 2	Ear vein.....	3 drops gelatin culture.	Found dead next morning.
Do.....	72	1,620 grams.	Jan. 2	....do	¼ cc. gelatin culture.	Died at end of 2½ hours.
Do.....	73	300 grams...	Jan. 3	Cavity of abdomen.	1 cc. gelatin culture.	Died at end of 7 hours.
Do.....	74	200 grams...	Jan. 3	....do	....do	Do.
Do.....	75	1,800 grams.	Jan. 3	....do	¼ cc. gelatin culture.	Result negative.
Do.....	76	1,750 grams.	Jan. 4	Ear vein.....	3 drops bouillon culture.	Do.
Do.....	77	2,000 grams.	Jan. 4	....do	....do	Do.
Do.....	79	....do	Jan. 8	Cavity of abdomen.	1 cc. gelatin culture.	Found dead at end of 20 hours.
Do.....	80	550 grams...	Jan. 8	....do	4 cc. bouillon culture.	Do.
Do.....	81	480 grams...	Jan. 8	....do	2 cc. bouillon culture.	Do.
Do.....	82	470 grams...	Jan. 8	....do	1 cc. bouillon culture.	Do.
Do.....	83	1,750 grams.	Jan. 8	....do	1 cc. gelatin culture.	Do.
Do.....	84	1,115 grams.	Jan. 9	....do	1 cc. bouillon culture.	Result negative.
Do.....	85	1,015 grams.	Jan. 9	....do	½ cc. bouillon culture.	Do.
Do.....	86	1,735 grams.	Jan. 15	....do	3 cc. gelatin culture.	Died at end of 7 hours.
Do.....	87	1,792 grams	Jan. 15	....do	....do	Died in 3 hours.
Do.....	88	1,770 grams.	Jan. 15	....do	½ cc. gelatin culture.	Died in 7 hours.
Do.....	89	1,887 grams.	Jan. 15	....do	....do	Died in 3 hours.
Do.....	90	2,430 grams.	Jan. 15	....do	....do	Found dead next morning.
Do.....	91	1,940 grams.	Jan. 15	....do	4 cc. gelatin culture.	Died in 3 hours.
			1888.			
Guinea pigs.	7	.....	May 28	....do	½ cc. bouillon culture.	Result negative.
Do.....	21	.....	Aug. 10	....do	1 cc. bouillon culture.	Do.
Do.....	22	.....	Aug. 10	....do	....do	Do.

Animal.	No.	Size.	Date.	Where injected.	Amount.	Result.
			1889.			
Guinea pig ..	23	.....	Aug. 13	Cavity of abdomen	8 cc. gelatin culture.	Died next morning.
Do.....	32	.....	Sept. 27	....do	2 cc. gelatin culture.	Do.
Do.....	33	.....	Sept. 27	....do	....do	Result negative.
White rats...	1	.....	Aug. 23	Subcutaneous	3 cc. bouillon culture.	Do.
Do.....	2	.....	Aug. 23	....do	5 cc. bouillon culture.	Do.
Do.....	3	.....	Aug. 23	....do	....do	Do.

These experiments show that the bacillus of Gibier is very pathogenic for rabbits when injected into the cavity of the abdomen in quantities of 1 cubic centimetre or more, that it is less pathogenic for guinea-pigs, and is not pathogenic for white rats when injected subcutaneously. Gelatine cultures seem to possess more intense pathogenic power than bouillon cultures, and cultures from the blood of an animal recently dead as the result of an inocculation are more potent than those from my original stock which had not been passed through a susceptible animal.

The mode of death in rabbits is quite characteristic. A couple of hours after receiving in the cavity of the abdomen 2 or 3 cubic centimetres of a liquefied gelatine culture the animal becomes quiet and indisposed to eat or move about. Soon after it becomes somnolent, the head drooping forward and after a time resting between the front legs with the nose on the floor of its cage. It can be roused from this condition, and raises its head in an indifferent and stupid way when pushed or shaken, but soon drops off again into a profound sleep. Frequently the animals die in a sitting position with their nose resting upon the floor of the cage between the front legs. I have not seen this lethargic condition produced by inoculations with any other microörganism. Convulsions sometimes occur at the moment of death.

The time of death depends upon the potency of the culture and its quantity as compared with the size of the animal.

With a full dose of 3 to 4 cubic centimetres of a liquefied gelatine culture death commonly occurs in from 3 to 7 hours.

The rapidity with which death occurs when a considerable quantity of a liquefied gelatine culture is injected into the cavity of the abdomen, and the somnolence which precedes death, give rise to the supposition that the lethal effect is due to the presence of a toxic chemical substance, rather than to a multiplication of the bacillus in the body of the animal. And this view is supported by the fact that animals frequently recover when the dose administered is comparatively small, and especially when it is injected subcutaneously. I have made a few experiments

with cultures sterilized by heat for the purpose of testing the truth of this supposition. The results are given below:

September 1, 1888.—Injected subcutaneously into rabbit No. 34, 15 cubic centimetres sterilized bouillon culture of Gibier's bacillus. Temperature at 11 a. m., before inoculation, 102.6°; temperature at 12:30, after inoculation, 103.5°; at 2 p. m., 104.3°; at 4 p. m., 105.8°; at 6 p. m., 105°. September 2, 9 a. m., temperature 104°. Animal appears well. Result negative except for the rise of temperature noted.

September 6, 12 *m.*—Injected into cavity of abdomen of small rabbit (weight 400 grammes), 5 cubic centimetres sterile gelatine culture of Gibier's bacillus. Found dead September 8, at 8 a. m. Liver dark-colored; stomach, spleen, and intestine normal.

September 4, 11:30 *a. m.*—Injected into cavity of abdomen of rabbit No. 35 (weight 400 grammes), 2 cubic centimetres sterilized gelatine culture of Gibier's bacillus. Result negative.

December 20, 1888.—Injected into cavity of abdomen of rabbit 66 (weight 972 grammes), 4 cubic centimetres sterilized gelatine culture of Gibier's bacillus, at 10 a. m. Temperature before injection, 101° F.; temperature at 1 o'clock, 101°. At 2:30 p. m. injected a second dose of 5 cubic centimetres sterilized culture into cavity of abdomen. At 3:30 the animal is somnolent. At 4:30 p. m. injected 4 cubic centimetres of same culture; temperature at 4:30, 100.5° F. At 5 p. m. the animal seems feeble and somnolent; temperature at 6 p. m., 102.2°. December 21, 10:30 a. m., the animal seems better. Injected into cavity of abdomen 5 cubic centimetres of the same sterile gelatine culture. December 29, the animal remains in good health and was injected in the cavity of the abdomen with 1 cubic centimetre liquefied gelatine culture, not sterilized, to test protective value of previous inoculations. Result of injection negative.

My time having been fully occupied with other experimental work I have not yet followed up this experiment upon the protective value of inoculations with sterilized cultures, but infer from the above experiment that the result of a careful experimental inquiry would establish the fact that a certain degree of immunity, at least, may be conferred by inoculations with culture fluids sterilized by heat.

The temperature of rabbits which receive a lethal dose of a culture of this bacillus falls below the normal sometime before death. This is shown by the following notes:

August 20, 10:45 *a. m.*—Injected subcutaneously into rabbit 32 (weight 420 grammes), 6 cubic centimetres bouillon culture of Gibier's bacillus. Temperature just before injection, 102° F.; temperature at 12 o'clock, 103°; at 1 o'clock, 103°; at 4 o'clock p. m., 99°. Animal appears feeble and dull; temperature at 6:15 p. m., 99°. Found dead next morning.

September 24, 10:30 *a. m.*—Two small rabbits, Nos. 51 and 52, injected in cavity of abdomen with 3 cubic centimetres liquefied gelatine culture of Gibier's bacillus. At 12:30 both animals are very lethargic and somnolent; temperature in rectum, 99°; temperature at 1:30 p. m., 95°. One died at 1:30 and one at 3 p. m., both in convulsions.

January 15, 1889, 10:30 *a. m.*—Injected into cavity of abdomen of rabbit No. 86 (weight 1,735 grammes), 3 cubic centimetres liquefied gelatine culture of Gibier's bacillus. At 2 p. m. the animal is lethargic; temperature, 99.8° F.; temperature at 2:45 p. m., 98°. Died in convulsions at 5:30 p. m. Temperature immediately after death in rectum, 96°.

January 15, 1889, 10:30 *a. m.*—Injected into cavity of abdomen of rabbit No. 88 (weight 1,770 grammes), one-half of a cubic centimetre gelatine culture bacillus *g*, from feces 56, Decatur (Gibier's bacillus). Temperature at 2 p. m., 100.2°; is lethargic. Temperature at 2:45, 98.5°. Dying at 5:30 p. m.; temperature, 96.6°. Died at 6 p. m.

In all cases in which death occurs, even when but a few hours have elapsed since the inoculation was made, I have recovered the bacillus in cultures made from blood obtained from the heart or the interior of the liver, and, as stated, these cultures appear to have a greater virulence than those not passed through the rabbit.

In sections of the liver and kidney stained with Loeffler's solution of methyline blue the bacilli are seen, and are often in rather long jointed filaments.

INJECTIONS INTO THE INTESTINE.

Upon my return from Havana in 1888 I admitted the possibility that this bacillus might have an etiological relation to yellow fever, although my culture experiments in 10 cases had only demonstrated its presence in the contents of the intestine 3 times. I therefore made a variety of experiments with it, and among others the following:

November 27, 1888, 3 *p. m.*—Injected into lumen of intestine of dog No. 2, small black and tan, 2 cubic centimetres liquefied gelatine culture of Gibier's bacillus. Animal died of peritonitis from the operation at end of 36 hours; liver and kidney normal.

November 27, 1888.—Injected into lumen of intestine of dog No. 4, 2 cubic centimetres liquefied gelatine culture of Gibier's bacillus. Temperature taken for 8 days showed no notable departure from the normal, and the animal made a good recovery.

December 4, 1888.—Injected into lumen of intestine of dog No. 5, 2 cubic centimeters liquefied gelatine culture of Gibier's bacillus. Temperature taken for 7 days showed no notable departure from the normal, and the animal remained well.

December 4, 1888.—Injected into lumen of intestine of dog No. 6, 2 cubic centimetres liquefied gelatine culture of Gibier's bacillus. Temperature taken for 7 days showed no notable departure from the normal, and the animal remained well.

December 18, 1888.—Injected into lumen of intestine of rabbit 69 (weight 720 grammes), one-half of a cubic centimetre liquefied gelatine culture of Gibier's bacillus. The result was negative. On the 29th of December 1 cubic centimetre, liquefied gelatine culture was injected beneath the skin of this rabbit. The animal was found dead next morning, still warm; diffuse cellulitis from point of inoculation; stomach and intestine enormously distended with gas; spleen slightly enlarged; intestine glued together and to walls of abdomen by adhesions at point of previous operation.

December 18, 1888.—Injected into lumen of intestine of rabbit No. 70 (weight 720 grammes), one-half of a cubic centimetre liquefied gelatine culture of Gibier's bacillus. Result negative.

The pathological appearances observed in rabbits which succumb to a lethal dose of a culture of this bacillus are not very marked; the spleen is sometimes enlarged, but otherwise the abdominal viscera appear to be normal. When the injection is made subcutaneously a diffuse cellulitis, extending from the point of inoculation, is frequently observed.

THE BACILLUS OF LACERDA AND BABES.

In 1883 Dr. Lacerda, of Rio de Janeiro, having discovered what he believed to be microörganisms in the liver and kidney of yellow-fever cases, sent some of the material to Paris, to Dr. Babes. This bacteri-

ologist demonstrated the presence of a microbe in this material and described it as follows:

The filaments appear united and homogeneous with an amplification of 600 diameters, but with a high power, one-twelfth hom. im., or No. 12 of Verick (which corresponds with the one-eighteenth of Zeiss), one can assure himself that these filaments are composed of elliptical grains, almost cylindrical, arranged in pairs, forming little groups in which they are united by an intermediary pale substance. The filaments are thus composed of diplococci, or, if one wishes, of very short rods with terminal spores.

Dr. Lacerda has described the organism referred to as in filaments which branch dichotomously, and believes this branching to be a constant and distinctive characteristic of the parasite, which he accepts as the veritable yellow-fever microbe. He is without doubt mistaken. The apparent branching of the filaments which he has described and drawn, and which he showed me in some of his preparations at the time of my visit to Rio, is due simply to the accidental juxtaposition of the torula-like chains. He is also mistaken in supposing that this organism is only to be satisfactorily demonstrated by Gram's method of staining. My friend, Dr. Goes, shared this belief at the time of my visit to Rio, but I demonstrated to him the facility with which the organism may be stained with a solution of methyline blue, upon sections which he made for me from material in Dr. Lacerda's laboratory. Since my return to Baltimore I have made numerous sections from the same material, and find no difficulty in staining the organism present in the tissues with methyline blue or with fuchsin.

Babes himself has renounced the idea that this microörganism bears an etiological relation to the disease under consideration. In the second edition of "Les Bactéries" he says:

Since these researches we have had the opportunity to examine several series of sections from yellow fever. First, the liver and kidney of two individuals dead from this malady, collected by Dr. Alvarez, were examined in the laboratory of pathological anatomy of the faculty of Paris, without any bacteria having been found; second, material from three cases of yellow fever which Koch was kind enough to confide to one of us. In these last three cases, notwithstanding the most scrupulous research, and notwithstanding the advice of Koch, it was impossible to find the little chains in the brain, the kidneys, the liver, and the spleen. We must suppose, then, that in yellow fever, as in other infectious maladies, microbes are only found in the parenchymatous organs in certain cases, and not in all. The question whether these microörganisms really constitute the cause of the malady, or simply a complication, is consequently not resolved.

The fact that this microörganism is not present in the liver and kidney of forty cases in which I have made autopsies in Havana, is ample evidence that its presence in the material from Dr. Lacerda's laboratory, which was sent to Babes, was accidental, and bore no relation to the etiology of the malady.

I shall submit, in connection with this report, a large series of slides showing thin sections of these organs stained by methods which demonstrate the presence of this bacillus in material containing it. Various

microörganisms are present in some of these cases and have been referred to elsewhere, but neither Dr. Councilman nor myself, after a careful search, have been able to find this bacillus in chains in the numerous sections examined by us. I shall submit at the same time a number of slides stained by the same methods, and showing thin sections from material brought by me from Dr. Lacerda's laboratory belonging to the cases in which this bacillus was first found, and in these the bacillus is well shown. There is, therefore, no question that its presence would have been demonstrated in sections from my Havana material stained in the same way if it were really present.

I insist upon this point because Dr. Frank Billings, in a letter dated March 6, 1889, published in the "Medical Register" of Philadelphia, states that he has found this bacillus in material from seven cases. He says:

> Suffice it to say that each has been carefully examined, and in each the same organism found as described by me, March last, and in the Register last year, and described by Babes in 1885, and in only one specimen is there any pollution, and that but slight, of a few specimens of a large bacillus.

Dr. Billings obtained his material through my friend Dr. D. M. Burgess, of Havana, and it so happens that the material from two of the six cases sent him by Dr. Burgess was from autopsies made by myself at the military hospital. At Dr. Burgess's request I permitted him to take material for Dr. Billings at these two autopsies which he assisted me in making. At the same time I preserved material in alcohol and in sealed tubes for making cultures, as was my practice.

The two cases referred to are those given in Dr. Billings' list as follows:

> 5. Soldier, died of yellow fever in military hospital, Havana, June 3, 1888. Autopsy 5 hours after death.
> 6. Soldier, died on June 6, 1888. Autopsy 3 hours after death.

These are the two last autopsies made by me in Havana, in 1888, and are numbered in my list 9 and 10. My notes of these cases are as follows:

> Autopsy No. 9, June 3, 1888: Soldier in military hospital; sick 5 days; autopsy 5 hours after death. Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder. Numerous colonies of bacillus *a* developed in cultures from blood, liver, urine, and kidney.
>
> Autopsy No. 10, May 6, 1888: Soldier in military hospital; sick 5 days; autopsy 1 hour and 40 minutes after death. (Dr. Burgess is mistaken in giving the time as 3 hours.) Collected material from liver, kidney, stomach, and intestine, blood from heart, and urine from bladder. No development in cultures from blood, liver, kidney, or urine.

Since my return from Cuba numerous sections have been made from the material preserved in alcohol from these two cases. These sections have been made by myself, by my assistant, Dr. Emilio Martinez, and by my friend Dr. James E. Reeves of Chattanooga.

The sections from case 9 show the same bacillus which was demonstrated to be present in great numbers in the blood, liver, and kidney by my cultures made from fresh material. This is my bacillus *a*, which by a careful research I have shown to be identical with the *bacterium coli commune* of Escherich.

As stated elsewhere, this is the microörganism which is most frequently present in yellow-fever tissues, as shown both by the culture method and in stained sections of material preserved in alcohol. It is longer than the Babes bacillus and is not united in chains. No doubt Dr. Billings found the same bacillus in his material from the same case, for it is present in very great abundance and is easily stained by the usual methods.

In case 10 my cultures gave a negative result, but in my sections from the kidney and in those mounted for me by Dr. Reeves there is present a micrococcus. This is an exceptional case in this regard, being the only one in which I have encountered micrococci. They are present in some of the sections only, in masses blocking up the capillaries. Dr. Billings seems not to have found them, as he says "in only one specimen is there any pollution, and that but slight, of a few specimens of a large bacillus."

Dr. Billings goes on to say:

> Furthermore, the organism is in the blood in every section and in great numbers, every authority to the contrary. Now I do hold, also against all contradiction, that in such a disease as yellow fever, where one finds one organism closely and sharply in many sections and all parts of these sections, and in all these sections but two specimens of another, that that organism is the cause of the disease of which the individual died. Here we have seven "undoubted cases of yellow fever," in which this one organism is present in immense numbers, so plentiful that I boldly say it is want of technical ability both as bacteriologists and microscopists that others have not found them. I will say further, that it is the same organism recently seen by Dr. Reeves, of Tennessee, but where his material came from I do not know, but I have one of his slides.

Is it then want of technical ability that has prevented Babes from finding a bacillus, first demonstrated by him in the Rio material, in other material carefully searched in order to confirm his discovery? I have already quoted what he says, but repeat the quotation:

> Since these researches we have had the opportunity to examine several series of sections of yellow fever; first, the liver and kidney of two individuals dead from this malady, collected by Dr. Alvarez, were examined in the laboratory of Pathological Anatomy of the Faculty of Paris, without any bacteria having been found; second, material from three cases of yellow fever which Koch was kind enough to confide to one of us. In these last three cases, notwithstanding the advice of Koch, it was impossible to find the little chains in the brain, the kidneys, the liver, and the spleen.

I may refer here to my researches prior to my visit to Brazil, and quote from my previous report:

> Desiring to supplement the observations made in Havana, in 1879, by further researches, I wrote to my friend Dr. Daniel M. Burgess, of Havana, some time during the summer of 1884, requesting him to obtain for me small pieces of liver, kidney,

and stomach from one or more typical cases of yellow fever. I made it an essential condition that the autopsies should be made within an hour or, at the outside, two hours after death, so that there might be no question of post-mortem changes. Small pieces of the organs named were to be put at once into a large quantity of strong alcohol. In compliance with my request Dr. Burgess obtained and forwarded to me material from two cases, which reached me in good condition, and upon microscopic examination the liver and kidneys showed the pathological changes constantly found in the disease in question. During the winter of 1884 I mounted numerous thin sections from this material, stained with various aniline colors. In none of them did I find any microörganisms, except upon the surface of the mucous membrane in sections of the stomach, where various organisms—bacilli and micrococci—were to be seen in properly stained sections. These were, however, only upon the surface, attached to the epithelium, or mingled with a granular débris adhering to the surface of the mucous membrane. In the autumn of 1885, during a visit to Dr. Koch's laboratory in Berlin, I had an opportunity to avail myself of the suggestions and valuable assistance of the master in bacteriology, and again studied the material which Dr. Burgess had sent me from Havana by the various methods of staining considered to be most useful in such a research. At the request of Dr. Koch I was assisted by Dr. Carl Seitz, who was at the time engaged upon his studies of the typhoid bacillus, and was an expert in staining and mounting thin sections of the tissues. Dr. Seitz and myself examined numerous sections of liver and kidney stained by various methods, with an entirely negative result so far as the presence of microörganisms was concerned. After my return to Baltimore, in 1886, I again made numerous sections from the same material and stained them with Lœffler's alkaline solution of methyline blue, which we had also used in Dr. Koch's laboratory, and with other aniline colors, but without any better success.

The following summer I went to Brazil, where I had no difficulty in demonstrating the presence of Babes bacillus in material containing it, preserved in Dr. Lacerda's laboratory, by exactly the same method of staining (Lœffler's solution) which had given me a negative result with the material examined in Koch's laboratory and in Baltimore.

Finally, Dr. Billings accords to Dr. Reeves of Chattanooga the technical skill necessary to demonstrate the presence of this bacillus. He says: "I will say further that it is the same organism recently seen by Dr. Reeves, of Tennessee, but where his material came from I do not know, but I have one of his slides."

This statement has induced me to place in Dr. Reeves hands material from a series of twenty-five cases. He has made for me from this material a series of slides which certainly exhibit technical skill of a high order; they are stained especially to demonstrate the presence of microörganisms, and various bacilli are present in those which represent certain cases. These I have described in the proper place, but they do not show the presence of the bacillus of Babes which Dr. Billings says is present in all his material "in every section and in great numbers."

At my request Dr. Councilman has gone over these slides of Dr. Reeves with great care and the result of his examination fully accords with my own. Moreover, Dr. Reeves' sections show bacilli in the same cases as do those mounted by myself and my laboratory assistant. They have no advantage over my own in the demonstration of microörganisms present.

With reference to the slide mounted by Dr. Reeves which Dr. Billings has seen, I have no doubt it is one of those made by him from my Decatur material. I made but three autopsies in Decatur, and the material preserved from these was placed in alcohol which was sent me in a tin can from a neighboring town. I have always suspected that this alcohol was not of sufficient strength to properly preserve the tissues. On my way home from Decatur I stopped over for a day to see my friend, Dr. Reeves, who begged me to give him some of my yellow-fever material for study. Upon opening the box containing it I found that one of the bottles was broken and the alcohol had escaped. I therefore left all of the material with Dr. Reeves, requesting him to place the fragments from the broken bottle into fresh alcohol, and to hold the whole subject to my order.

To make a long story short, Dr. Reeves found in sections from one of these cases bacilli in great numbers, which were photographed for him by Dr. Detmers, of Ohio. The sections containing those bacilli contained also other bacilli and micrococci. The bacillus present in greatest abundance resembles in its morphology my bacillus *a*; it certainly is not the bacillus of Babes.

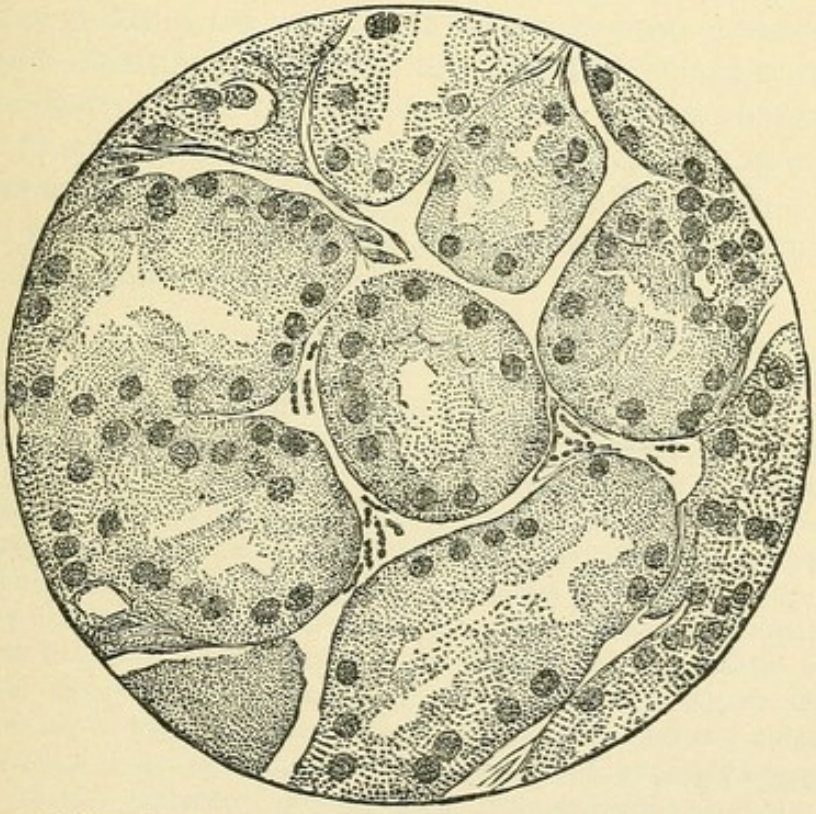


FIG. 21.—Bacillus of Babes in kidney, yellow fever. Material from Dr. Lacerda's laboratory in Rio Janeiro.

The morphology of the bacillus of Babes and its distribution in the tissues are shown in Fig. 21, which is taken from my article on yellow fever in Wood's Handbook of the Medical Sciences. The bacillus is magnified about 1,000 diameters, while the amplification for the tissue elements is 450.

Dr. Babes' drawings of the same bacillus will be found on p. 525 of his work, "Les Bactéries," Paris, 1886, 2d edition.

All pathological experts to whom I have shown a duplicate of the section from which Dr. Detmers' photographs were made agree with me that the microörganisms present represent a post-mortem invasion of the tissue, and of the particular piece of tissue from which the section was made. The outer margin of the piece is evidently invaded by putrefactive organisms. Whether this piece came from the broken bottle I can not say, but I attach no importance to the finding of microörganisms in this material under the circumstances mentioned, and I have excluded these Decatur cases from further consideration, as I have an abundance of material in which I have greater confidence from the autopsies made in Havana in 1888 and 1889.

VIII.—DESCRIPTION OF MICROÖRGANISMS ISOLATED FROM YELLOW-FEVER CADAVERS, AND THE ALVINE DISCHARGES OF THE SICK.

BACILLI.

No. 1: Bacterium coli commune (Escherich). My bacillus *a*.

This is the bacillus which I have obtained most frequently in my cultures from the blood and tissues, and which has been present most constantly and abundantly in my plate cultures (Esmarch roll-tubes) in flesh-peptone-gelatine from material obtained post mortem from the stomach and intestine, and from the alvine discharges of the sick. Being a facultative anaërobic, it has also been the microörganism most constantly found in my anaërobic cultures in glycerine agar.

The characters of this bacillus are given as follows in a paper read by the writer at the meeting of the Association of American Physicians held in Washington in September, 1888:

Bacillus *a* is from 1½ to 4 times as long as broad, and has a diameter of about 0.6 of a micromillimetre; the dimensions vary considerably, however, in different cultures, and even in the same culture. Under certain circumstances the ends of the rods are seen to be more deeply stained than the central portion. This appearance is not, however, constant, and seems to depend upon the age of the culture, and upon the time during which it is subjected to the action of the staining reagent. The ends of the rods are more or less rounded, and they are often associated in pairs.

Young colonies in gelatine (Esmarch tubes) have a pale straw color which afterward becomes pale brown; they are spherical and homogeneous, or lobate. Colonies which come to the surface often present the appearance of a rosette, or of a flower with its petals expanded, as in the daisy. From cultures in which lobate colonies and rosettes were the prevailing form I have obtained in a second series of Esmarch tubes, made from a single typical rosette, colonies which were not lobate, and which did not form rosettes (spherical straw-colored homogeneous colonies); and in these cultures superficial colonies when young have the appearance of a fragment of broken glass; later they present an irregular margin, and a more or less wrinkled appearance of the interior. These are the characters of the bacillus *f* found as the prevailing form in my cultures from the stomach and intestine in my last two cases.

It is a very curious fact that during the last week of my stay in Havana I failed entirely to obtain my bacillus *a* in lobate colonies and rosettes, although I have three times repeated the experiment of making a series of Esmarch tubes from a single

typical colony in form of rosette in Esmarch tubes preserved from my earlier cases. In every instance I have had instead colonies which are identical with those in other Esmarch tubes inoculated at the same time from single colonies of my bacillus *f* of case 9 and case 10. As the morphology of the bacilli is also the same I am forced to the conclusion that bacillus *a* and bacillus *f* are one and the same, and that the character which during my earlier experiments I found to be constant—*i. e.*, the colonies in form of a rosette—is not so, but that under certain circumstances, at present undetermined, this bacillus forms lobate colonies and rosettes, and under other conditions homogeneous spherical colonies in the deeper portion of the gelatine, and broken glass colonies upon the surface. Later these colonies have an irregular, vitreous-looking margin, and the interior has a brown color, more or less intense.

In my cultures made in 1889 the *bacterium coli commune* was again the bacillus most frequently encountered, but the rosette colonies found so commonly in my earlier culture experiments in 1888 were not observed except in one or two instances.

My identification of my bacillus *a* with the *bacterium coli commune* of Escherich is the result of a careful study of cultures in various media, made side by side with it. I quote again from my paper above referred to:

Since my return to Baltimore I have given much time to the elucidation of the question referred to. It is evident that if identity is once established with any known bacillus found elsewhere than in connection with yellow fever, the microorganism can be excluded as the possible specific cause of this disease. But the present status of bacteriology makes it also evident that great care must be taken in assuming the identity of microörganisms from different sources, and that morphological characters or even similarity of growth in culture media will not alone suffice. Any constant difference in physiological characters, as for example in pathogenic power when tested on various animals, must be accepted as establishing a specific form, or a permanent variety, which amounts to the same thing. We now know, for example, two spirilla which resemble closely the spirillum of Asiatic cholera (the Finkler-Prior spirillum and the "cheese spirillum" of Deneke), but which have, nevertheless, been shown by carefully conducted experiments to be different in certain particulars which would easily escape notice upon a superficial comparison.

I have felt that with this knowledge to guide me I could not lightly exclude any microörganism as common, and especially that a bacillus which was so prominent by reason of its constant and abundant presence should receive careful consideration.

Two bacilli have been described, both of which are found in the intestines of healthy individuals, which in their morphology resemble my bacillus *a*, found in the alimentary canal of yellow-fever cases. These are the *Bacillus Neapolitanus* of Emmerich and the *Bacterium coli commune* of Escherich. Both of these microörganisms have been the object of extended studies by German bacteriologists, and especially the bacillus of Emmerich, on account of his claim that it is the specific etiological agent in the disease with which he found it associated. This claim has not been substantiated; on the other hand, it has been shown by Weisser* that "in human feces, normal as well as abnormal, in the air and in putrid material, bacteria are found, which in their morphology, their biological functions, and their pathogenic action upon animals are identical with the so-called Naples cholera-bacterium of Emmerich."

I have had for comparison a culture of the Emmerich bacillus, originally from one of the bacteriological laboratories of Germany, preserved in the collection main-

* Ueber die Emmerich'schen sogenannten Neapler Cholerabacterien. Zeitschrift für Hygiene, Band 1.

tained under the direction of Prof. William H. Welch in the pathological laboratory of Johns Hopkins University. For a culture of the bacterium coli commune I am indebted to Dr. William D. Booker, of Baltimore, who had it directly from Escherich. The characters of both of these microörganisms have been carefully defined, but I find no account of the lobate colonies (deep), or of the superficial colonies in the form of rosettes, which became for me, during my earlier experiments in Havana, the diagnostic character by which I recognized my bacillus *a* in gelatine Esmarch tubes. By lobate colonies, I mean that instead of being spherical and homogenous, the colonies are divided into a small number of distinct masses without losing their spherical outline. In some cases this apparent segmentation of the colonies extended further, and they became coarsely granular in appearance. The superficial colonies in the form of rosettes also varied considerably, the margins being sometimes made up of symmetrical lobes, shaped like the petals of a daisy—marguerite—and at others being more deeply cleft and dentate. If this character of growth had proved to be constant it would suffice to differentiate my bacillus *a* from the two microörganisms in question. But it proves not to be constant, and since my return to Baltimore I have only obtained these rosette colonies exceptionally, and have not been able to determine the precise conditions which determine their formation. But I have ascertained that, cultivated side by side in the same media and under the same conditions of temperature, the colonies formed in gelatine Esmarch tubes are identical in the case of my bacillus *a*, and the colon bacillus of Escherich. The growth upon agar-agar and upon potato is also identical, and the reducing power upon litmus added to sterilized milk.

My gelatine cultures have been made in flesh-peptone gelatine containing 20 per cent. of gelatine, instead of 10 per cent. as in Koch's original formula. I have found this to stand the summer heat of Baltimore without liquefaction, and during the months of May and June used it with great satisfaction in Havana for Esmarch tubes and stick cultures. It will stand a temperature of about 68° F. (30° C.), and so far as I can see answers as a culture medium for diagnostic purposes quite as well as that containing 10 per cent. of gelatine.

The idea which I had entertained that isolated colonies in Esmarch tubes should give me pure cultures of a single organism has proved not to be true in many instances in my first series of tubes inoculated with material from the intestine or stomach, and it seems probable that such colonies frequently originate from little masses of associated microörganisms, rather than from a single bacillus. In making gelatine stick cultures in 20 per cent. gelatine from single Esmarch colonies of my bacillus *a*, I have frequently had, both in Havana and since my return to Baltimore, an outgrowth at intervals along the line of puncture, which is shown in Fig. 1 of the plate accompanying this paper (Plate V). This consists of feathery tufts growing out into the gelatine. To test the question whether these tufts represent a different microörganism or a different mode of growth of the same, I have several times carefully broken the test tube containing a growth like that shown in Fig. 2, and have made a stick culture in gelatine from the feathery tuft and also from that part of the stick which was free from these outgrowths; one from the tuft has been a similar growth all along the line of puncture, as shown in Fig. 3; the other has resembled precisely that portion of the original stick culture which was free from these outgrowths. Having, in Havana, made this differentiation, I designated the tufted growth bacillus *l*. The morphology of the bacillus when cultivated in bouillon or obtained from the surface of an agar or gelatine culture appeared to be identical with that of bacillus *a*; but preparations mounted directly from the feathery tufts showed that, together with short oval bacilli of the typical form, there were numerous long filaments like that shown at *a*, in Fig. 6. A very curious thing about my stick cultures of this bacillus *l*, made in Havana, was that after a few days they assumed a brownish color, which in time became black.

In cultivating Emmerich's bacillus in 20 per cent. gelatine at a temperature of 28° to 30° C. (82.4° to 86° F.) I have obtained the same radiating feathery growth as is presented by my bacillus *l*. This is shown in Fig. 4, which, like the other figures illustrating this paper, is copied from a photograph made by myself. This mode of growth, so far as I know, has not been described, but I have no reason to doubt the authenticity of the stock from which the culture was made. The growth upon potato and the morphology correspond with the descriptions of those who have specially studied this bacillus, and with my bacillus *l*. I am therefore forced to the conclusion that they are identical. The morphological characters of this bacillus are shown in Fig. 6, which is made from a bouillon culture of bacillus *l*, separated, as heretofore stated, while in Havana, from a stick culture of my bacillus *a* (*Bacterium coli commune* of Escherich).

I must now call attention to Fig. 5. This is from a stick culture in 20 per cent. gelatine of the colon bacillus of Escherich, obtained from him, as heretofore stated, through Dr. Booker, of Baltimore. We have here a feathery outgrowth along the line of puncture, which appears to be identical with that shown in Fig. 1 from my bacillus *a*. To test the question of identity I broke this tube and made a stick culture in 20 per cent. gelatine from the tuft at *a*, and another from the clear portion of the column above this point. The result was a growth in the first tube like that from which the inoculation was made, similar to Figs. 3 and 4, and in the second tube the characteristic growth of the colon bacillus, viz, spherical, light brown colonies crowded together into a central core at the upper part of the puncture, but more or less distinct and separate at the lower extremity. Thus it will be seen I have had the same result from this authentic culture of the colon bacillus which was sent by Escherich to Dr. Booker, and had been passed through a series of Esmarch tubes by the last-named bacteriologist, as that obtained in Havana and since in Baltimore from stick cultures of my bacillus *a*. This forces me to one of two conclusions: Either the colon bacillus of Escherich and the bacillus of Emmerich may remain associated after passing a culture containing them through a succession of plate cultures (Esmarch tubes) or the so-called bacillus of Emmerich (my bacillus *l*) is a variety of the colon bacillus—a sport which is produced under certain circumstances, and which retains its distinctive character for a certain time. I am not prepared at present to decide this question in a definite manner, but propose to keep it in view in my future studies of these microörganisms.

I have since obtained the same tufted outgrowth in gelatine stick cultures from single Esmarch colonies of my bacillus *a* in cultures from feces made in Decatur, Ala., and from the contents of the stomach and intestines of yellow-fever cadavers, made in Havana in 1889. Although not constant, this outgrowth is so common in my stick cultures in 20 per cent. gelatine that I have come to look upon it as one of the distinguishing characters of the colon bacillus.

Flügge speaks of this bacillus as having "*geringe Beweglichkeit*," and Eisenberg in his Bakteriologische Diagnostik, says: "Träge beweglich." I have never been able to convince myself that the colon bacillus, either in my cultures from yellow-fever cadavers, etc., or from Escherich's stock, obtained by me from Dr. Booker, has any proper vital movements. I have observed in hanging drop cultures motion *in situ*, which I take to be molecular, although it is not as active as the molecular movements of inorganic particles are often observed to be. It is rather a gentle swinging motion of the little rods in the direction of their

long axis, and is not attended with a change of location of individual rods with reference to others in the same field.

While in Havana and since my return to Baltimore I have made numerous inoculation experiments in rabbits and guinea pigs, with cultures of this bacillus. The general results obtained may be briefly stated as follows:

The injection into the cavity of the abdomen of a considerable quantity of a bouillon culture, for a full grown rabbit from 3 to 5 cubic centimetres, causes a decided elevation of temperature, which may amount to more than 2° C. (3.6° F.), and death usually occurs within 2 to 4 days. A certain proportion of the animals, however, survive these large doses, and in smaller amounts (less than 2 cubic centimetres) recovery is the rule. That the febrile movement is due to a ptomaine produced during the active development of the microörganism is indicated by the fact that cultures sterilized by heat (70° C., 158° F.) for 10 minutes likewise give rise to a temporary rise of temperature.

In fatal cases in which nonsterilized cultures were injected into the cavity of the abdomen, I have recovered the bacillus from the blood and tissues, in pure cultures, and these possess pathogenic properties identical with those shown by the original cultures.

The number of bacilli present in the blood and liver is comparatively small, as is shown by the examination of stained smear preparations.

The most striking post-mortem appearance in animals which succumb to an intraperitoneal injection is a rosy hyperæmia of the small intestine. The liver is dark in color, full of blood, and rather soft. The spleen is normal in appearance.

These results show that in its pathogenic properties, as well as in its morphology and growth in culture media, my bacillus *a* corresponds with the bacterium coli commune of Escherich, and consequently that it is not the specific etiological agent in yellow-fever.

The following is a partial record of experiments made:

May 15, 1888, 1 *p. m.*—Injected into cavity of abdomen of rabbit No. 2, one-half cubic centimetre bouillon culture of bacillus *a*. Result negative.

May 15, 1:30 *p. m.*—Injected into cavity of abdomen of rabbit No. 3, 2 cubic centimetres bouillon culture bacillus *a*; 3:20 p. m., temperature 104.5°; May 16, 7 a. m., temperature 106°; 3 p. m., 104.5°; May 20, apparently well; injected into cavity of abdomen 3 cubic centimetres bouillon culture bacillus *a*. Found dead at 6 a. m., May 24.

May 15, 1 *p. m.*—Injected into cavity of abdomen of rabbit No. 4, 2 cubic centimetres bouillon culture of bacillus *a*. Temperature at 3:30 p. m., 104°; May 16, at 7 a. m., temperature 105.5°; 3 p. m., 104.5°; May 17, 7 a. m., appears better. May 21, 7 a. m., found dead.

May 20.—Injected into cavity of abdomen of rabbit No. 5, 2 cubic centimetres bouillon culture bacillus *a*; found dead on the morning of May 25.

May 18, 12:30 *p. m.*—Injected into cavity of abdomen of rabbit No. 7, 15 cubic centimetres sterilized bouillon culture of bacillus *a* (sterilized by heat at 160° F.). Temperature at 4 p. m., 40.9° C.; at 8 p. m., 40.4° C.; May 19, 6 a. m., temperature 39.6° C.; 12m., 39.4°; 4 p. m., 39.8°; 20, 6 a. m., temperature 39.5° C. At 12 m., May 20, injected subcutaneously 2 cubic centimetres bouillion culture of bacillus *a* and the same amount into the cavity of the abdomen—to test protection by sterilized culture. May 21, 6 a. m., temperature 39.2°; found dead on the morning of May 25.

May 18, 12:30 *p. m.*—Injected subcutaneously into rabbit No. 8, 5 cubic centimetres sterile culture of bacillus *a*; temperature just before injection 39.6°; temperature at 4 p. m., 40.6°. Second injection of 5 cubic centimetres sterile culture made at 4 p. m., temperature at 8 p. m. 40.7°. May 19, 6 a. m., temperature 41°, gave a third dose of

3 cubic centimeters sterile culture; 12 m., temperature 40°, injected a fourth dose of 3 cubic centimeters sterile culture; 4 p. m., temperature 41.1°, injected 3 cubic centimeters sterile culture; 8 p. m., temperature 41.6°, injected 3 cubic centimeters sterile culture. May 20, 6 a. m., temperature 40.5°, injected 5 cubic centimetres sterile culture; 12 m., temperature 39.2°. Died at 4 p. m. May 21, liver large and rather soft; spleen, normal; intestine, normal.

May 22, 12 *m.*—Injected into a small rabbit, No. 9, 2 cubic centimetres mixed bouillon and agar cultures, bacillus *a*; result, negative.

May 22, 12 *m.*—Injected into a small rabbit, No. 10, 2 cubic centimetres of a mixed bouillon and agar culture, bacillus *a*; found dead next morning.

May 31.—Injected into cavity of abdomen of rabbit No. 13, 3 cubic centimetres bouillon culture of bacillus *a*; found dead at 6 a. m. June 2; liver dark in color and rather soft, stomach and intestine normal.

July 26, 1889, 7 *a. m.*—Injected into cavity of abdomen of rabbit No. 128, 4 cubic centimetres culture in *agua coco* of bacillus *a* from yellow fever feces No. 3. July 27, 3 p. m., the animal is quite feeble; 28, 6 a. m., found dead; peritonitis.

May 13, 6:15 *p. m.*—Injected subcutaneously into guinea-pig No. 1, 2 cubic centimeters bouillon culture bacillus *a*. May 14, 7 a. m., appears sick and declines food; May 15, appears better; May 16, eats a little; May 17, appears dull; May 18, died at 8 a. m. Liver appears to be of a lighter color than usual (fatty?); bladder, empty; stomach, empty; large intestine filled with a dark liquid, bloody, resembling that sometimes found in intestine of yellow fever cadavers; reaction slightly acid. Bacillus *a* recovered in cultures from blood of heart and from liver.

May 18, 12 *m.*—Injected subcutaneously into guinea-pig No. 2, 2 cubic centimetres bouillon culture bacillus *a*; 4 p. m., temperature 39.2°; 8 p. m., 38.7°. May 19, 6 a. m., temperature 38.7°, animal appears sick; 12 m., temperature 39.5°; 4 p. m., temperature 39.2°. May 20, 6 a. m., temperature 38.4°; 12 m., 38.3°. May 21, 12 m. 38.7°. May 22, 6 a. m., temperature 38.2°; animal appeared well and was used for another experiment.

May 18, 12 *m.*—Injected into guinea-pig No. 3, 10 cubic centimetres sterilized culture of bacillus *a*; 5 cubic centimetres in cavity of abdomen, and 5 cubic centimetres subcutaneously. Temperature in rectum immediately after injection 39.2° C.; 4 p. m., 38.2°; 8 p. m., 38.3°; May 19, 6 a. m., temperature 38.6°; 12 m., 38.6°; 4:30 p. m. 39.4°; May 20, 6 a. m., temperature 39.8°; 12 m., 39.2°; May 21, 12 m., 38.8°; May 22, remains well.

May 18, 12 *m.*—Injected into cavity of abdomen of guinea-pig No. 4, one-half a cubic centimetre bouillon culture bacillus *a*. Temperature immediately after injeccion 39.6°; 4 p. m., temperature 40.5°; 8 p. m., 39.6°; May 19, 6 a. m., temperature 38.6°; 12 m., 39.3°; May 20, 6 a. m., temperature 39.5°; May 21, 12 m., temperature 38.9°; the animal remained in good health.

Upon my return from Havana, in 1888, I was still uncertain whether the little tufts developed along the line of puncture in gelatine stick cultures of my bacillus *a* were to be considered a "wuchsform" of this bacillus, or an associated bacillus which was specifically distinct. As already stated I made cultures from these tufts by breaking the test-tube, and designated this bacillus *l*. The following experiments upon animals have been made with pure cultures of the bacillus obtained in this way:

Baltimore, July 6, 1888.—Injected subcutaneously into rabbit No. 25, 4 cubic centimetres bouillon culture of bacillus *l*. Result negative.

Baltimore, September 1, 1888.—Injected subcutaneously into rabbit No. 33, 15 cubic centimetres sterilized bouillon culture bacillus *l*. Result negative.

Baltimore July 10, 1888.—Injected into cavity of abdomen of guinea-pig No. 8, 1, cubic centimetre bouillon culture of bacillus *l*. July 12, 3 p. m., the animal was very sick and was killed. Peritonitis; small intestine hyperæmic; liver dark in color; spleen slightly enlarged.

Baltimore, July 10, 1888.—Injected into cavity of abdomen of guinea-pig No. 9, 4 cubic centimetres bouillon cultures of bacillus *l*. Animal found dead morning of July 13. Small intestine hyperæmic; liver dark in color; gall bladder distended with a clear fluid.

Baltimore, July 16, 1888.—Injected subcutaneously into guinea-pig No. 10, one-half cubic a centimetre bouillon culture bacillus *l*. Result negative.

Baltimore, July 16, 1888.—Injected subcutaneously into guinea-pig No. 11, 2 cubic centimetres bouillon culture bacillus *l*. Result negative.

Baltimore, July 16, 1888.—Injected into cavity of abdomen of guinea-pig No. 12, one-half a cubic centimetre bouillon culture, bacillus *l*. Result negative.

Baltimore, July 16, 1888.—Injected into cavity of abdomen of guinea-pig No. 13, one-half a cubic centimetre bouillon culture, bacillus *l*. Result negative.

Baltimore, July 21, 1888.—Injected into lumen of intestine of small black dog, 3 cubic centimetres bouillon culture bacillus *l*. Result negative.

Baltimore, July 21, 1888.—Injected into lumen of intestine of large dog, 4 cubic centimetres bouillon culture bacillus *l*. Result negative.

These experiments will suffice to show that this bacillus does not produce, in the animals experimented upon, any symptoms or pathological changes which can be identified with those of yellow fever in man.

NO. 2. BACILLUS *x* (HAVANA, 1889.)

The general results of my culture experiments in 1888 having enabled me to exclude the supposition that the specific infectious agent in the disease under investigation is a liquefying microörganism, I have naturally turned my attention to the nonliquefying bacilli present in the alimentary canal, and in certain cases obtained in my cultures from the tissues. The one most constantly and abundantly present, my bacillus *a*, having been excluded, I have given much time to the study of other nonliquefying bacilli associated with it, and especially to that one which I have designated by the letter *x*, and which, for the present, I shall give no other name.

This bacillus resembles the *bacterium coli commune* (bacillus *a*) in its morphology, although somewhat larger; and its colonies in gelatine roll-tubes are also quite similar, especially when young. It is, however, fully differentiated from the "colon bacillus" by its pathogenic power when injected into the peritoneal cavity of rabbits.

I am now satisfied that this bacillus was present in my cultures made from the intestine of yellow-fever cadavers in 1888, although I did not differentiate it from my bacillus *a* at that time. This is shown by the pathogenic potency of certain cultures supposed to contain only bacillus *a*, while pure cultures in bouillon, made from single colonies, proved not to be pathogenic. The apparently contradictory results obtained in my inoculation experiments I could not at the time explain, but now believe this to be the true explanation. I first recognized this bacillus

by its pathogenic power, in experiments starting from material obtained from the liver of case 18 (autopsy May 13, 1889). Three minims of this material, containing also the large anaërobic bacillus designated by the letter N in my experiments made at this date, was injected into guinea pig No. 43. Death occurred at the end of 14 hours. A second guinea pig (No. 45) was inoculated with 3 minims of bloody serum from the subcutaneous connective tissue of the first. At the end of 48 hours there was a pouch in the abdominal walls containing a collection of bloody serum. A little of this was drawn off in a capillary tube, and cultures made from it. It was in these cultures that I first recognized my bacillus *x*. Its great pathogenic power when injected into the cavity of the abdomen of rabbits was first demonstrated by the following experiment:

Havana, May 22, 12 *m.*, 1889.—Injected into the cavity of the abdomen of rabbit No. 108, 1 cubic centimetre culture of bacillus *x* in glycerine agar. At 2:30 p. m. the animal is lying upon its side breathing slowly, and is evidently dying; died at 2:40 p. m.

Other experiments, showing the virulence of cultures of this bacillus, will be given later. My object at present is to point out the fact that it is differentiated from bacillus *a* by this pathogenic power.

In my subsequent researches I have obtained this bacillus in about one-half the cases, either directly in my cultures from material contained in the small intestine, or indirectly in my inoculation experiments upon rabbits and guinea pigs. It does not follow that it was not present in those cases in which I have not demonstrated its presence. My autopsies at this time followed each other in quick succession, and a complete bacteriological study of each case was impracticable. If the colonies in gelatine roll-tubes had presented well-marked differences from those of the colon bacillus, the matter would have been greatly simplified; but I did not feel justified in attempting to decide that bacillus *x* was present in such tubes from an examination of the colonies alone, or from this and the examination of a stained preparation together. Nothing short of the inoculation of a pure culture into the cavity of the abdomen of a rabbit seemed to me at that time to suffice for the differentiation. The difficulty is increased by the fact that the colonies of both bacilli vary considerably in the same medium at different times. In general, however, the deep colonies of bacillus *x* are more opaque, and of a deeper brown color than those of the colon bacillus, and the superficial colonies are thicker and more opaque. The difficulties referred to prevent me also from estimating with any degree of accuracy the relative abundance of bacillus *x* in the contents of the intestine. I do not hesitate to say, however, that the colon bacillus has been the most constant and most abundant microörganism in my cultures from this source. I have more frequently obtained bacillus *x* in my inoculation experiments than in cultures made directly from the contents of the intestine or material from the liver kept in an anti-

septic wrapping. It has been frequently present in cultures made from the liver of animals which have died from such inoculations.

CHARACTERS OF BACILLUS *x*.

This bacillus varies considerably in its *morphology*, as is shown by my photomicrographs. In recent gelatine cultures it is often so short an oval in form that it might be mistaken for a micrococcus. In cultures in bouillon or in cocoanut water it resembles the colon bacillus, but is larger—1μ or more in diameter. The rods are often united in pairs, and in the same culture may vary considerably in length (Fig. 3, Pl. VI). In potato cultures they are seldom seen as long as in Fig. 2, Pl. VI, which is exceptional in this regard, and led to the suspicion that another bacillus had, by accident, taken possession of the sterilized potato on which bacillus *x* had been planted. But upon making gelatine roll tubes from this culture it proved to be a pure culture of bacillus *x*, and upon replanting it on another potato presented its usual form. In my photomicrograph from this potato culture it will be observed that some of the rods present the appearance of not being filled out at the ends, and others show a faint line at the extremity, which seems to include a vacuole, or unstained extremity of the rod. This is an appearance which I have frequently noticed not only in potato cultures, but in those in various liquid media. When stained preparations are examined with the full light of the Abbe condenser, the ends of some of the rods appear to be cut away, leaving a concave extremity; but by using a small diaphragm to obtain definition it will be seen that the cell wall extends beyond the stained portion of the rod and includes what appears to be a vacuole. There is no reason to believe that this appearance is due to the presence of an end spore, for the supposed vacuole is not refractive, as a spore would be, and my experiments on the thermal death point of this bacillus indicate that it does not form spores. Cultures are sterilized by exposure for 10 minutes to a temperature 160° F. (71.2° C.).

A very curious thing, with reference to this bacillus, is that it presented active movements in my cultures made directly from yellow-fever cadavers, but that these movements were not constant, and that since my return to Baltimore I have not, as a rule, observed active movements in cultures from the same stock, which, however, preserved their pathogenic power and other characters. In Havana these movements were usually not observed in all the bacilli in a field under observation, but one and another would start from a quiescent condition on an active and erratic course; sometimes spinning actively upon its axis, and again shooting across the field as if propelled by a flagellum.

My notes indicate that cultures passed through the guinea-pig are more apt to be motile, but my attention had not been called to this until now that I am engaged in writing them out. Thus I find recorded in my Havana notes "Single colonies from guinea-pig No. 136 the

bacillus is actively motile; also from gelatine stick culture of 2 days made from single colony; also motile in cultures in cocoanut water at times; at other times not. The movements are rapidly progressive or rotatory."

This culture from guinea-pig No. 136 was one of the stock cultures which I brought home with me, and which has served for my experiments in Baltimore. It came through a series of inoculated guinea pigs from the liver of case 28, as follows:

Havana, July 16, 7:30 *a. m.*, 1889.—Injected subcutaneously into guinea-pig 153 5 minims blood and crushed parenchyma from liver of case 28 * containing bacillus N., etc. At 12 m. the animal is running about the cage in the usual restless and eccentric manner. Died at 10 p. m. Extensive effusion of bloody serum, with separation of the skin over abdominal walls; various bacilli in this bloody serum.

July 17, 8 *a. m.*—Injected subcutaneously into guinea-pig 154 5 minims of bloody serum from cellular tissue of guinea-pig 153. Convulsions at 8:30 a. m., July 18. Died at 12:30. Extensive subcutaneous œdema and softening of tissues. Liver rather dark in color, spleen somewhat enlarged. Culture from liver large motionless bacillus (N), and smaller motile bacillus (*x*).

July 18, 5 *p. m.*—Injected into guinea-pig 157 4 minims of bloody serum from connective tissue of guinea-pig 154. July 20: The animal has an extensive collection of bloody serum in the walls of the abdomen (some of this was drawn off and injected into guinea-pig 160, which died at end of 20 hours). July 21: The animal has been very sick, but seems better. Killed at 5 p. m., July 21; liver dark in color, abdominal viscera normal.

July 20, 7:30 *a. m.*—Injected subcutaneously 4 minims of bloody serum drawn during life, from subcutaneous connective tissue of guinea pig No. 157 into guinea pig No. 160. Died at 12:30, July 21. No subcutaneous œdema, fibrinous deposit on surface of liver, which is rather light in color, stomach hyperæmic. Obtained bacillus *x* in cultures from liver.

It is from this source, and from another series, started from case 18, that my experiments with bacillus *x* have been made.

Turning now to my Baltimore notes I find: "Recovered bacillus *x* from spleen and liver of guinea-pig 190, and spleen of 189. In this culture in cocoanut water, at the end of 24 hours, bacillus *x* is actively motile, as in my first cultures in Havana."

These two guinea pigs received, by subcutaneous injection, on the 17th of December, 1889, 1 cubic centimeter of a culture of bacillus *x* in cocoanut water, one week old. The experiment was made to see if bacillus *x* could be recovered after an interval of several days. The animals remained in apparent good health (bacillus *x* alone does not kill guinea pigs when injected subcutaneously). On the 23d of December the animals were killed and cultures made, with the usual precautions, from their liver and spleen.

All of these contained bacillus *x* in pure cultures, and actively motile, as in Havana. With this exception I have not observed active movements in my numerous cultures of this bacillus since my return to Baltimore. That it was the veritable *x* which I recovered from the

* This is a case in which the autopsy was made 9 hours after death, and smear preparations from the liver showed the presence of bacilli.

above-mentioned guinea-pigs was demonstrated by making cultures in gelatine roll tubes, etc.

In gelatine stick cultures the growth of bacillus *x* resembles that of the colon bacillus, but the colonies at the bottom of the line of puncture are more opaque and not of a clear amber color like that of colonies of the colon bacillus. Upon the surface the growth is thicker than that of the colon bacillus, and forms a milk white, soft mass, Fig. 7, Pl. VI.

The tufted outgrowth observed so frequently in gelatine stick cultures of my bacillus *a* does not occur in similar cultures of this bacillus.

This bacillus is a *facultative anaërobic.*

It grows well in agar cultures and especially in *glycerine agar*, in which it produces some gas and an acid reaction. The growth on the surface of glycerine agar cultures is white, cream-like in consistency, and quite abundant.

It grows well in an agar or gelatine medium, made acid by the addition of 0.2 per cent. (1:500) of hydrochloric acid.

In cocoanut water it multiplies rapidly, producing a milky opacity of the previously transparent fluid, an acid reaction, and an evolution of carbon dioxide.

On potato it produces a thick layer, which may cover the entire surface in three or four days, and which has a dirty-white, cream-white, or pinkish-white color, and cream-like consistency. The growth upon potato varies at different times, evidently owing to differences in the potato. This is shown by my notes with reference to the potato culture from which my photomicrograph was made, Fig. 2, Pl. VI.

In potato culture, slide 1681, negative 573, bacillus *x* has grown as a thin cream-white layer; the rods are longer than usual, and have vacuoles at the ends as observed in Havana. Esmarch roll-tubes from this potato show it to be a pure culture with typical colonies, one of which (slide 1689) shows elements of various lengths, some short oval. Another potato culture from the above gives the usual short oval form. The growth of the first potato with long form is more scanty than usual, and white in color; the potato is not discolored; another potato inoculated from this one turns bluish, the growth is abundant and of a yellowish-white color. The first potato has an acid reaction, the second is neutral or slightly alkaline.

The colonies in gelatine Esmarch roll-tubes vary considerably at different times. Deep colonies are usually spherical, homogeneous, light-brown in color, and more opaque than the similar colonies of the colon bacillus. At the end of a few days the deep colonies become quite opaque and may be lobate like a mulberry, or coarsely granular; sometimes the deep colonies have an opaque central portion surrounded by a transparent marginal zone.

In old gelatine roll-tubes these deep colonies form opaque, white hemispheres, projecting from the surface of the dried culture medium, and little tufts of acicular crystals are sometimes observed to project from the side of such old colonies.

The superficial colonies are circular or irregular in outline, with transparent margins and an opaque central portion, sometimes corrugated. They are finely granular and iridescent by reflected light, and of a

milk-white color; by transmitted light they have a brownish color. Young colonies closely resemble those of the colon bacillus (*a*). This bacillus grows well at a temperature of 20° C. (68° F.), but more rapidly and luxuriantly at a higher temperature, 30° to 35° C. Its vitality is not destroyed by exposure in a freezing mixture of ice and salt for 2 hours. The thermal death point is between 140° and 145° F. (60° to 62° C.), but to insure the sterilization of cultures contained in test-tubes or flasks I am in the habit of subjecting them to temperature of 160° F.

Bacillus *x* is pathogenic for rabbits when injected into the cavity of the abdomen.

This is shown by the following experiments arranged in tabular form:

INJECTED INTO CAVITY OF ABDOMEN.

Rabbit No.	Weight in grams.	Date.	Culture.	Amount.	Result.
		1889.			
108	small	May 22	Glycerine agar	1 cc	Died at end of 2 hours and 40 minutes.
109	large	May 24	Agar culture	1 cc	Result negative.
111	1,000	June 5	Veal broth	10 cc	Died at end of 5½ hours.
120	.....	June 27	Agua coco	1 cc	Died at end of 11 hours.
121	.....	June 29	....do	1½ cc	Recovered.
125	large	July 25	...do	3 cc	Died at end of 5 hours.
126	medium	July 25	....do	4 cc	Died at end of 2 hours.
127	...do	July 26	....do	3 cc	Died at end of 3 hours.
132	.....	July 28	....do	4 cc	Died at end of 6½ hours.
157	1,500	Aug. 8	...do	2½ cc	Died at end of 9½ hours.
166	1,500	Aug. 12	....do	3 cc	Died at end of 22 hours.
190	1,100	Nov. 16	....do	5 cc	Died at end of 2½ hours.
192	1,350	Nov. 18	....do	3 cc	Died at end of 4 hours.
193	1,200	Nov. 19	....do	1 cc	Died at end of 2 hours.
208	1,425	Dec. 13	....do	1½ cc	Died at end of 3 hours.
		1890.			
216	1,425	Jan. 3	....do	1 cc	Recovered.
221	930	Jan. 13	...do	3 cc	Dead next morning.
222	910	Jan. 13	....do	3 cc	Dead morning of Jan. 15 (48 hours).
223	1,250	Jan. 31	Blood serum	2 cc	Dead at end of 3 hours.
224	1,330	Jan. 31	....do	2 cc	Dead next morning (22 hours).
234	945	Feb. 26	....do	1½ cc	Do
235	1,090	Feb. 26	....do	½ cc	Do
244	1,300	Mar. 3	....do	2 cc	Do
255	1,250	Mar. 10	Bouillon	5 cc	Dead at end of 3 hours.
259	1,520	Mar. 10	....do	8 cc	Found dead next morning.
260	1,710	Mar. 10	....do	10 cc	Do
293	2,270	Apr. 2	....do	1½ cc	Died at end of 30 hours.

SUBCUTANEOUS INJECTIONS INTO RABBITS.

		1889.			
109	large	May 20	Gelatine culture	3 drops	Result negative.
110	...do	May 24	Agar culture	2 drops	Do.
112	.....	June 5	Veal broth	¼ cc	Do.
119	.....	June 27	Agua coco	1 cc	Died at end of 30 hours.
		1890.			
265	1,520	Mar. 13	Bouillon	2 cc	Result negative.

INJECTIONS INTO EAR VEIN OF RABBITS.

		1889.			
210	1,260	Dec. 17	Agua coco.....	2 minims..	Result negative.
		1890.			
218	1,180	Jan. 8	Blood serum.....	3 minims..	Do.
219	1,575	Jan. 8	Agua coco.....	4 minims .	Do.

The negative results obtained in injecting cultures beneath the skin or into the ear vein of rabbits show that this bacillus does not induce a fatal septicæmia in these animals, and the fatal result when injections are made into the peritoneal cavity does not appear to be due to an invasion of the blood, but rather to the local effects upon the peritoneum together with the toxic action of the chemical products resulting from its growth.

It is true that I have always been able to recover the bacillus from the liver, or from blood obtained from one of the cavities of the heart, even in animals which succumb within a few hours to an injection made into the cavity of the abdomen. But the direct examination of the blood shows that the bacilli are present in very small numbers, and leads me to believe that the bacillus does not multiply, to any considerable extent at least, in the circulating fluid.

The spleen is not enlarged, as is the case in anthrax, rabbit septicæmia, and other diseases in which the pathogenic micro-organism multiplies abundantly in the blood.

On the other hand there is evidence of local inflammation in the peritoneal cavity. When death occurs within a few hours the peritoneum is more or less hyperæmic and there is a considerable quantity of straw-colored fluid in the cavity of the abdomen. When the animal lives for 24 hours or more there is a decided peritonitis with a fibrinous exudation upon the surface of the liver and intestine.

In the above table it will be seen that the most of the animals injected in the cavity of the abdomen died within 24 hours, but I shall shortly give some experiments in which death occurred at a later date as a result of a partial protection from previous injections made. In these cases the results of a fibrinous peritonitis, with adhesions, are clearly seen. I have spoken of the exudation upon the surface of the liver and intestines in animals which die at the end of 20 hours or more as "fibrinous." Under the microscope it is seen to be composed largely of leucocytes, and these commonly contain bacilli in their interior, as is seen in my photo-micrograph, Fig. 1, Pl. VI. Whether a genuine phagycytosis occurs I have not yet definitely determined, but propose to give my attention to the question hereafter. In one case, in which an animal was killed some days after receiving an injection into the peritoneal cavity, there was evidence of fibrinous peritonitis, and the liver was cirrhotic. In several other cases the liver has also seemed to me to be harder than normal, but no definite evidence of recent connective tissue

growth has been made out in thin sections of the organ. Usually the liver in animals which die within 24 hours is full of blood, rather soft, and dark in color. In a single instance I found the liver to be of a light color, and loaded with fat. As the animal was excessively fat and this was an exceptional case, I have not supposed that the observation is entitled to any special weight in estimating the evidence with regard to this bacillus from an etiological point of view.

The rapidly fatal effect in those cases in which I have injected 2 or more cubic centimeters of a culture into the cavity of the abdomen has led me to suppose that death results from the toxic effects of a ptomaine contained in the culture at the time of injection. The symptoms also give support to this supposition. The animal quickly becomes feeble and indisposed to move, and some time before death lies helpless upon its side, breathing regularly, but is too feeble to get up on its feet when disturbed. Death sometimes occurs in convulsions, but more frequently without, apparently from heart failure.

My experiments upon guinea-pigs are given in the following table. They show that this bacillus is less pathogenic for these animals than for rabbits.

Experiments on guinea-pigs.

No.	Date.	Culture.	Amount.	Where injected.	Result.
	1889.				
66	May 29	Agua coco	½ cc.	Subcut	Negative.
78	June 3	....do	1 cc.	....do	Very sick, but recovered.
104	June 13	Glyc. agar	½ cc.	....do	Result negative.
119	June 24	Agua coco	1 cc.	....do	Dead at 6 a. m. next morning.
121	June 26	... do	½ cc.	....do	Sick 2 days, but recovered.
155	July 17	....do	½ cc.	....do	Dead next morning.
162	July 21	...do	½ cc.	....do	Result negative.
163	July 22	....do	1 cc.	....do	Do.
168	Nov. 16	....do	½ cc.	....do	Do.
169	Nov. 18	....do	2 cc.	Cavity of abdomen....	Dead at end of 20 hours.
170	....do ...	...do	3 cc.	....do	Do.
171	Nov. 20	....do	¼ cc.	Subcut	Result negative.
172	....do ...	...do	½ cc.	.. do	Do.
189	Dec. 17	....do	1 cc.	....do	Do.
190	....do ...	....do	1 cc.	....do	Do.
	1890.				
204	Mar. 10	Bouillon	6 cc.	Cavity of abdomen ...	Dead at end of 18 hours.
205	....do ...	... do	2 cc.	....do	Do.

I have made the following experiments on dogs:

Baltimore, November 16, 10:30 *a. m.*, 1889.—Injected into cavity of abdomen of small puppy, 3 months old, 2 cubic centimetres of a culture of bacillus *x* in agua coco. At 2 p. m. the dog appeared quite sick and indisposed to move and continued so during the afternoon. Temperature in rectum at 4 p. m., 104° F. November 17, 9 a. m., the dog appears well; temperature, 100.2°. November 18th, continues well; temperature, 101°. On this date, at 10:30 a. m., injected into cavity of the abdomen 5

cubic centimetres culture of bacillus *x* in agua coco. Temperature at 3:30 p. m., 102.4°. Dog appears lively. Next day apparently well.

Baltimore, November 19, 10:30 *a. m.*—Injected into cavity of abdomen of small puppy, 5 months old, 4 cubic centimetres culture of bacillus *x* in agua coco. Temperature just before injection, 102.4°; temperature at 3 p. m., 103.8°; the animal is evidently sick and lies quietly in its box. November 20, 10 a. m., seems better, but still quiet; temperature, 102.6°. November 21, jumps about and appears perfectly well.

EXPERIMENTS WITH CULTURES STERILIZED BY HEAT.

Havana, July 26, 1889.—Injected into cavity of abdomen of rabbit 129, 4 cubic centimetres sterile culture bacillus *x* in agua coco. Result negative.

July 28, 1889.—Injected into cavity of abdomen of rabbit No. 130, 8 cubic centimetres sterile culture of bacillus *x* in agua coco. Result negative.

July 30, 1889.—Injected into cavity of abdomen of rabbit 137 8 cubic centimetres sterile culture bacillus *x* in agua coco. Result negative.

July 30, 1889.—Injected into cavity of abdomen of rabbit 138 10 cubic centimetres sterile culture bacillus *x* in agua coco. Result negative.

August 21, 1889.—Injected into cavity of abdomen of rabbit 182, weight 1,000 grams, 7 cubic centimetres sterile culture bacillus *x* in agua coco. Result negative.

These experiments show that the death of animals which have received in the cavity of the abdomen 2 or 3 cubic centimetres of a non-sterilized culture of bacillus *x* is not due alone to the toxic action of a ptomaine present in the culture, but depends upon the presence of the living bacilli, unless in the process of sterilization at 160° F. the ptomaine has been destroyed. The latter supposition is worthy of attention, and the question may be determined experimentally by injecting cultures from which the bacilli have been removed by filtration.

I have made a few experiments with a view to determining whether animals which have received full doses of sterilized cultures have any subsequent immunity from the effects of non-sterilized cultures.

Baltimore, Nov. 28, 1889.—Injected into cavity of abdomen of rabbit 198 6 cubic centimetres sterile culture bacillus *x* (animal weighs 1,000 grams). December 11, 9 a. m., injected into cavity of abdomen 2 cubic centimetres culture bacillus *x* in agua coco. The animal died at the end of 7 hours after receiving this injection.

Baltimore, March 4, 1890.—Injected into cavity of abdomen of rabbit No. 274, weight 1,480 grams, 5 cubic centimetres sterile culture bacillus *x* in bouillon with 5 per cent glycerine. March 10, 9 a. m, animal in good health, weighs 1,375 grams. Injected into cavity of abdomen 4 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. of glycerine. Dead next morning at 8 o'clock.

March 6, 1890.—Injected into cavity of abdomen of rabbit 251, weight 2,170 grams, 10 cubic centimetres sterile culture bacillus *x* in bouillon with 5 per cent. glycerine. March 10, 9 a. m., the animal appears well; weight 1,750 grams. Injected in cavity of abdomen 5 cubic centimetres culture bacillus *x* with 5 per cent. glycerine. Died in convulsions at 8.30, March 12.

March 13, 1890.—Injected into cavity of the abdomen of rabbit 266, weight 640 grams, 4 cubic centimetres sterile culture bacillus *x* in bouillon with 5 per cent. glycerine. March 18, 9.30 a. m., in good health; weight 610 grams. Injected into cavity of abdomen 2 cubic centimetres bouillon culture bacillus *x*. March 22, 10 a. m., in good health; weight 610 grams. Killed and abdominal viscera found to be normal in appearance.

March 13, 1890, 12 *m.*—Injected into cavity of abdomen of rabbit 267, weight 645

grams, 4 cubic centimetres sterile culture bacillus *x* in bouillon with 5 per cent. glycerine. March 18, remains well; weight 610 grams. Injected into cavity of abdomen 2 cubic centimetres bouillon culture bacillus *x* with 5 per cent. glycerine. Animal died at 3 p. m. next day.

March 10, 1890.—Injected into cavity of abdomen of rabbit No. 282, weight 1,100 grams, 12 cubic centimetres sterile culture bacillus *x*, in bouillon with 5 per cent. glycerine. March 22, 11 a. m., remains in good health; weight 1,030 grams. Injected in cavity of abdomen 5 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. glycerine. Found dead next morning; intense fibrinous peritonitis.

It will be seen that these experiments give somewhat contradictory results; some of the animals survived a lethal dose of a nonsterilized culture after having received a full dose of a sterile culture, and others did not. But in those which succumbed the time of death appears to have been delayed. Thus in experiment of March 13 a small rabbit which received at noon 2 cubic centimetres of a nonsterilized culture did not die until 3 o'clock the next day, while another rabbit of about the same size survived the injection of a similar amount (March 13). In another experiment (March 6) a large rabbit received a dose (5 cubic centimetres) which usually kills within 3 or 4 hours, but died at the end of 48 hours.

Still the experiments as a whole do not give satisfactory evidence of immunity as a result of the injection of sterilized cultures, and I am inclined to look upon the partial immunity afforded as due rather to *a tolerance on the part of the peritoneum* than to a general tolerance of the toxic products present in cultures of this bacillus. This view is supported by the fact that I have had even more decided evidence of immunity from the previous injection into the cavity of the abdomen of other bacilli.

This is shown by the following experiments:

March 10, 1890.—Injected subcutaneously into rabbit 258 1 cubic centimetre liquefied gelatin culture of bacillus pyocyanus. March 22, 10 a. m. weight 2,085 grammes; injected into cavity of abdomen 2 cubic centimetres liquefied gelatin culture of bacillus pyocyanus. March 27, 2 p. m., in good health, weight 1,675 grammes; injected into cavity of abdomen 4 cubic centimetres culture of bacillus *x* in bouillon with 5 per cent. glycerin. April 2, 11 a. m., weight 1,735 grammes; injected into cavity of abdomen 4 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. of glycerin. No result.

March 10.—Injected into cavity of abdomen of rabbit No. 255, weight 2,120 grammes, 10 cubic centimetres sterile culture of bacillus acidiformans. March 18, 10 a. m., weight 1,300 grammes; injected into cavity of abdomen 5 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. of glycerin. March 22, weight 1,290 grammes; thin and rather weak; killed; some evidence of peritonitis; liver apparently cirrhotic.

March 15.—Injected into cavity of abdomen of rabbit No. 275, weight 550 grammes, one-half cubic centimetre liquefied gelatin culture of bacillus 36. March 22, weight 630 grammes; injected into cavity of abdomen 1 cubic centimetre culture bacillus *x* in bouillon with 5 per cent. glycerin. March 27, remains well; weight 750 grammes; injected into cavity of abdomen 2 cubic centimetres bouillon culture of bacillus *x*. Found dead on the morning of March 29.

NOTE.—As a rule, 2 cubic centimetres of the culture of bacillus *x* would kill a rabbit of this size within 4 hours. We have, therefore, evidence of a certain degree of tolerance as a result of the previous injections.

March 17, 1890.—Injected subcutaneously into rabbit No. 276, weight 515 grammes, 1 cubic centimetre bouillon culture of bacillus gracilis. March 22, in good health; injected into cavity of abdomen 2 cubic centimetres culture bacillus gracilis in bouillon. March 27, weight 560 grammes; injected into cavity of abdomen 2½ cubic centimetres culture bacillus *x* in bouillon with 5 per cent. glycerin. Animal remains in good health April 20.

March 18.—Injected subcutaneously into rabbit 277, weight 1,740 grammes, 1½ cubic centimetres bouillon culture clostridium cadaverinus. March 22, weight 2,010 grammes; injected into cavity of abdomen 2 cubic centimetres bouillon culture bacillus gracilis. March 27, weight 1,890 grammes; injected into cavity of abdomen 3 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. of gelatine. April 2, weight 1,700 grammes; injected in cavity of abdomen 4 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. glycerin. April 4, in good health; killed at 11 a. m.; some fibrinous exudation upon surface of liver, intestine, and spleen; cirrhosis of liver.

March 18, 1890.—Injected subcutaneously into rabbit No. 279, weight 690 grammes, 3 cubic centimetres bouillon culture of bacillus pyocyanus. March 22, 11 a. m., weight 780 grammes; injected into cavity of abdomen 2 cubic centimetres liquefied gelatin culture bacillus pyocyanus. March 27, 1 p. m., weight 795 grammes; injected into cavity of abdomen 3 cubic centimetres culture of bacillus *x* in bouillon with 5 per cent. glycerin. Animal died at 1 p. m., March 28.

March 18, 1890.—Injected into cavity of abdomen of rabbit No. 280, weight 940 grammes, 5 cubic centimetres sterile culture of bacillus gracilis in bouillon. March 22, weight 1,060 grammes; injected into cavity of abdomen 3 cubic centimetres bouillon culture of bacillus gracilis (not sterilized). March 27, weight 1,155 grammes; injected into cavity of abdomen 2 cubic centimetres culture of bacillus *x* in bouillon with 5 per cent. of glycerin. April 2, weight 1,060 grammes; injected into cavity of abdomen 4 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. glycerin. April 4, in apparent good health; killed at 10 a. m.; no peritonitis, no development in gelatin stick culture from liver.

NOTE.—A larger rabbit, weight 2,270 grammes, succumbed to an injection of 1½ cubic centimetres of the same culture, injected on the 2d of April, at the end of 29 hours. In this animal the autopsy revealed an intense fibrinous peritonitis.

March 31, 1890.—Injected into cavity of the abdomen of rabbit No. 283, weight 1,220 grams, 1 cubic centimetre culture bacillus gracilis in bouillon. April 4, 11 a. m., weight 1,140 grammes; injected into cavity of abdomen 4 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. of glycerin. Found dead at 8 o'clock next morning; some peritonitis and considerable fluid in cavity of abdomen.

March 31, 1890.—Injected into cavity of abdomen of rabbit 284, weight 1,560 grammes, 1 cubic centermetre bouillon culture of bacillus gracilis. April 4, 11 a. m. weight 1,425 grammes; injected into cavity of abdomen 4 cubic centimetres culture bacillus *x* in bouillon with 5 per cent of glycerin. Animal survived injection.

March 31.—Injected into cavity of abdomen of rabbit 285, weight 910 grammes, 1½ cubic centimetres bouillon culture bacillus gracilis. April 4, 11 a. m., weight 980 grammes; injected into cavity of abdomen 4 cubic centimetres culture of bacillus *x* in bouillon with 5 per cent. of glycerin. Animal died on fourth day after injection. April 8, at 2 p. m., no peritonitis.

March 31, 1890.—Injected subcutaneously into rabbit 286, weight 1,280 grammes, 2 cubic centimetres culture of bacillus gracilis in bouillon. April 4, 11 a. m., weight 1,240 grammes; injected into cavity of abdomen 4 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. of glycerin. Animal found dead on the morning of April 11; no peritonitis. Bacillus of rabbit septicæmia obtained in culture from liver.

NOTE.—It is probable that the preceding rabbit also died from rabbit septicæmia which at this date carried off a number of rabbits kept in the same cage, among others, three which had injected in the cavity of the abdomen a sterilized culture of

bacillus pyocyanus. Pure cultures of bacillus caniculacida were obtained from the blood of all of these.

I have made a few experiments to determine whether any protection is afforded by subcutaneous injections or injections in the ear vein. These are given below.

December 17, 1889.—Injected into the ear vein of rabbit No. 210, weight 1,260 grammes, 2 drops of culture bacillus *x* in agua coco. January 3, 10 a. m., injected into cavity of abdomen 1½ cubic centimetres culture of bacillus *x* in agua coco; February 3, 10 a. m., weight 850 grammes, very thin and feeble; injected into cavity of abdomen 2 cubic centimetres culture of bacillus *x* in blood serum. Dead next morning.

December 17, 1889.—Injected into the ear vein of rabbit 211 4 drops of culture of bacillus *x* in agua coco. January 3, 10 a. m., injected into cavity of abdomen 1 cubic centimetre culture bacillus *x* in agua coco. Found dead on the morning of January 8 (5 days). No peritonitis, no bacilli found in liver.

January 8, 1890.—Injected into ear vein of rabbit 218, weight 1,180 grammes, 3 drops of culture of bacillus *x* in blood serum. Februry 3, 10 a. m., weight 1,710 grammes, injected into cavity of abdomen 2 cubic centimeters culture of bacillus *x* in blood serum kept in oven at 20° C. for 20 days. February 6, 10 a. m., weight 1,600 grammes; injected into cavity of abdomen 5 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. glycerine in oven at 35° C. for 3 days. Dead next morning at 8 o'clock.

March 13, 1890.—Injected into ear, not in vein but into connective tissue, 5 drops of a bouillon culture of bacillus *x*. March 18, 10 a. m., weight 2,010 grams; injected into cavity of abdomen 3 cubic centimetres bouillon culture bacillus *x*. Dead next morning at 8 o'clock.

March 13, 1890.—Injected subcutaneously into left side of rabbit 265, weight 1,520 grammes, 2 cubic centimetres bouillon culture of bacillus *x*. March 18, in good health, weight 1,450 grammes injected into cavity of abdomen 4 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. of glycerine. March 22, 10 a. m., in good health, weight 1,320 grammes. Killed, some fibrinous peritonitis.

In the following experiment a rabbit which survived the injection of 1 cubic centimetre in the cavity of the abdomen succumbed at a later date to a larger quantity:

January 3, 1890.—Injected into the cavity of the abdomen of rabbit 216, weight 1,425 grammes, 1 cubic centimetre of a culture of bacillus *x* in agua coco. February 3, 10 a. m., the animal has had an abscess in the middle of belly and is now very thin, weight 980 grammes; injected into cavity of abdomen 2 cubic centimetres culture bacillus *x* in blood serum. Died at 12 m., February 4. Adhesions of intestine from old peritonitis; considerable fluid in cavity of abdomen; liver very small and rather dark in color.

Experiment of March 13 gives evidence of immunity resulting from a subcutaneous injection, but more experiments are necessary to establish this point.

The experiments in this series do not support the idea that an infectious disease is produced in the rabbit by inoculating it with cultures of bacillis *x*, but a partial immunity from the effects of intra-peritoneal injections appears to result from previously introducing the bacillus into the circulation.

As already stated the evidence favors the view that death results from peritonitis (and toxæmia?) induced by intra-peritoneal injections, and that *a tolerance on the part of the peritoneum may be established by*

the injection of cultures of certain other bacilli, or of sterilized cultures of bacillus x.

I have also made a number of experiments with a view to determine the effects of temperature upon the virulence of cultures of this bacillus.

FREEZING DOES NOT DESTROY VIRULENCE.

February 5, 1890, 10 *a. m.*—Injected into cavity of abdomen of rabbit No. 228, weight 775 grammes, 2 cubic centimetres culture of bacillus *x* in blood serum, frozen for 2 hours in ice and salt mixture. Dead next morning at 8 o'clock. Bacillus *x* in pure culture recovered from liver.

February 5, 1890, 10 *a. m.*—Injected into cavity of abdomen of rabbit 229, weight 810 grammes, 2 cubic centimetres culture bacillus *x* in blood serum, frozen 2 hours in ice and salt mixture. Dead next morning at 8 o'clock. Bacillus *x* in pure culture recovered from liver.

For the following experiments the cultures were made at a temperature of 20° C.

January 31, 1890, 9:30 *a. m.*—Injected into cavity of abdomen of rabbit 223, weight 1,250 grammes, 2 cubic centimetres culture bacillus *x* in blood serum, No. 5 of a series cultivated at intervals of 3 days in incubating oven at 20° C. Found dead on return to laboratory at 12:30 a. m.

February 26, 1890, 9:30 *a. m.*—Injected into cavity of abdomen of rabbit 234, weight 945 grammes, 1½ cubic centimetres culture bacillus *x* in blood serum, No. 8 of a series at 20° C.; culture was 22 days old at time of injection. Animal dead next morning at 8 o'clock.

February 26, 1890, 9:30 *a. m.*—Injected into cavity of abdomen of rabbit 335, weight 1,090 grammes, one-half cubic centimetre culture bacillus *x* in blood serum (same culture as used for rabbit 234), culture No. 8 of series at 20° C., 22 days old. Animal dead next morning at 8 o'clock.

For the following experiments the cultures were made at a temperature of 35° C.:

January 3, 1890, 9:30 *a. m.*—Injected into cavity of abdomen of rabbit No. 229, weight 1,330 grammes, 2 cubic centimetres culture bacillus *x* in blood serum, No. 5 of series at 35° C., culture 3 days old. Animal found dead next morning at 8 o'clock. Pure culture of bacillus *x* from fluid in peritoneal cavity.

February 3, 1890.—Injected into cavity of abdomen of rabbit No. 227, weight 955 grammes, 2 cubic centimetres culture of bacillus *x* in blood serum, No. 3 of series at 35° C., culture 12 days old. Animal survived injection, and on March 5 weighed 1,340 grammes. At 10 a. m. this date injected into cavity of abdomen 4 cubic centimetres culture bacillus *x* in bouillon with 5 per cent. glycerine, 3 days in oven at 35° C. Animal dead next morning at 8 o'clock.

February 27, 1890.—Injected into cavity of abdomen of rabbit 232, weight 920 grammes, 1½ cubic centimetres culture bacillus *x* in blood serum, No. 8 of series at 35° C., culture 22 days old. Animal survived injection, and on March 6 weighed 850 grammes. 10 a. m. injected into cavity of abdomen 4 cubic centimeters culture bacillus *x* in bouillon with 5 per cent. of glycerine. Found dead next morning at 8 o'clock.

February 27, 1890, 9:30 *a. m.*—Injected into cavity of the abdomen of rabbit No. 233, weight 895 grammes, 1 cubic centimetre culture bacillus *x* in blood serum, No. 8 of series at 35° C. (same as used for rabbit 232), culture 22 days old. Animal died at 7:30 p. m.

The experiments in this series indicate that virulence is diminished by keeping a culture for some time at 35° C., but show that the animals which survived an intra-peritoneal injection with these attenuated cultures are not protected from the lethal effects of a recent culture.

I would say finally with reference to this bacillus that I have not encountered it in my comparative researches made by the same methods upon cadavers from other diseases than yellow fever.

In a single instance I obtained bacillus *x* in a culture from the liver of guinea pig 179, inoculated with 3 drops of material from the liver of a case of tuberculosis kept 48 hours in an antiseptic wrapping.

The animal died on the sixth day after receiving the inoculation and I recovered from the effused serum in the subcutaneous connective tissue, a bacillus which presented all the characters of bacillus *x*. A culture of this bacillus in agua coco killed rabbit 205 in 7 hours (3 cubic centimetres) and rabbit 207 in 4 hours (2½ cubic centimetres). This seemed to exclude bacillus *x* from further consideration as the possible etiological agent in yellow fever; but upon referring to the history of this guinea pig I found that it had been inoculated a week previously with a culture of bacillus *x* (one-half cubic centimetres injected subcutaneously, November 20, at 10 a. m.).

It will be remembered that the guinea pig is not killed by subcutaneous injections of cultures of this bacillus. To test the question as to whether bacillus *x* could survive for some days in the tissues of an inoculated guinea pig, I made the following experiments:

December 17, 1889, 9 *a. m.*—Injected subcutaneously into guinea pig No. 189 1 cubic centimetre culture bacillus *x* in agua coco. The animal remained in apparent good health, and was killed at 11 a. m. December 23. Cultures were made from its liver and spleen; in both bacillus *x* was obtained in pure culture.

The experiment was made in duplicate, 1 cubic centimetre of the same culture having been injected into guinea pig 190. The animal was killed at the same time, and cultures made from the liver and spleen contained bacillus *x*.

No 3. Bacillus acidiformans.—(My bacillus *i* of 1888.)

This bacillus I first obtained in June, 1888, from a piece of yellow-fever liver which had been preserved for 48 hours in an antiseptic wrapping; it was associated with bacterium coli commune and my bacillus cadaverinus. I have since obtained it from liver preserved in the same way in two of my comparative autopsies.

It is, therefore, excluded as far as the etiology of yellow fever is concerned, but as it is pathogenic and has some interesting characters I have given some time to its study.

The morphology of this bacillus is shown by my photomicrograph, Fig. 1, Pl. VII. It is larger than my bacillus *x*, and like it differs considerably in its dimensions at different times; the diameter may be as much as 1½ *μ*. Usually it has the appearance of a short rod, one and a half to two times as long as broad, but the elements are often oval in

form. It is *not motile.* Like the colon bacillus and bacillus *x* it is a *facultative anaërobic*, and like them it grows freely in an acid medium—flesh-peptone-gelatine containing 0.2 per cent. of hydrochloric acid. In cultures containing glycerine or sugar it produces an abundant evolution of carbon dioxide and a volatile acid is formed. Bouillon cultures to which 5 per cent. of glycerine has been added, and which have been carefully neutralized, acquire a decidedly acid reaction as a result of the growth in them of this bacillus, but without the addition of glycerine no acid is formed. I therefore infer that it has the power of breaking up glycerine, and have named it *bacillus acidiformans.* In cocoanut water, which contains glucose, it produces an intensely acid reaction and an abundant evolution of carbon dioxide.

It does not liquefy gelatine, and in stick cultures grows abundantly both on the surface and along the line of puncture. At the end of 24 hours at 22° C. a rounded white mass is formed upon the surface resembling the growth of Friedlander's bacillus; at the bottom of the line of puncture the separate colonies are spherical, opaque, and pearl-like by reflected light. Gas bubbles are formed in the gelatine. (See Fig. 8, Pl. VII.) At the end of a week the surface is covered with a thick white semi-fluid mass.

In gelatine roll tubes the superficial colonies are translucent or opaque and spherical or somewhat irregular in outline; by reflected light they are slightly irridescent; the deep colonies are spherical, opaque, and homogeneous.

The growth upon the surface of *nutrient agar* is abundant and rapid, of a shining milk-white color, and cream-like in consistency. An abundant development forms along the line of puncture and the culture medium is split up by gas bubbles. In glycerine agar the evolution of gas is very abundant and the culture medium acquires an intensely acid reaction.

On potato the growth is abundant and rapid at a temperature of 20° to 30° C., forming a thick, semi-fluid mass of a milk-white color.

I have not obtained any evidence that this bacillus forms spores; the cultures are sterilized by 10 minutes' exposure to a temperature of 160° F.

When cultivated in bouillon to which 5 per cent. of glycerine has been added the culture medium acquires a milky opacity and there is a copious precipitate, of a viscid consistency, consisting, of bacilli; during the period of active development the surface is covered with gas bubbles as in a saccharine liquid undergoing alcoholic fermentation, and the liquid has a decidedly acid reaction.

Pathogenic for rabbits and guinea pigs.—This is shown by the following experiments:

Animal.	Date.	Culture.	Amount.	Where injected.	Result.
	1888.				
Guinea pig ..	Aug. 31	Potato.....	Small ...	Cavity of abdomen ...	Died at end of 27 hours.
Do.....	Aug. 31	....do	... do	....do	Recovered.
Rabbit	Sept. 3	Bouillon	2 cc	....do	Died on fourth day.
Guinea pig ..	Sept. 1	....do	2 cc	....do	Died at end of 16 hours.
Do.....	Sept. 4	....do	3 cc	....do	Died at end of 40 hours.
Rabbit	Sept. 6	....do	1½ cc	....do	Died at end of 16 hours.
Do.....	Sept. 7	....do	1 cc	Subcutaneous	Died on seventh day.
Do.....	Sept. 7	....do	½ cc	....do	Do.
Do.....	Sept. 7	....do	¼ cc	Cavity of abdomen ...	Died on fifth day.
Guinea pig ..	Sept. 11	....do	2 cc	....do	Died at end of 28 hours.
	1889.				
Rabbit.....	Nov. 12	Agua coco...	1 cc	....do	Recovered.
Do.....	Dec. 24	...do	1½ cc	....do	Do.
Do.....	Dec. 24	....do	1 cc	....do	Died at end of 28 hours.
	1890.				
Do.....	Feb. 15	....do	1 cc	....do	Recovered.
Do.....	Feb. 28	Bouillon	2 cc	....do	Died at end of 9 hours.
Do.....	Mar. 1	Blood serum	1½ cc	....do	Died at end of 22 hours.
Do.....	Mar. 1	....do	1 cc	....do	Do.
Do.....	Mar. 3	Bouillon	2 cc	....do	Died at end of 20 hours.
Do.....	Mar. 3	Blood serum	2 cc	....do	Do.

The following experiments have been made with *sterilized cultures* of this bacillus:

Animal.	Date.	Culture.	Amount.	Where injected.	Result.
	1890.				
Rabbit.....	Mar. 4	Bullion	5 cc.	Cavity of abdomen.....	Negative.
Do.....	Mar. 10	....do	10 cc.	....do.....	Do.
Do.....	Mar. 10	....do	10 cc.	....do.....	Do.
Do.....	Apr. 1	....do	10 cc.	... do.....	Do.
Do.....	Apr. 1	....do	10 cc.	....do.....	Do.

In animals which succumb to an intra-peritoneal injection of a culture of this bacillus the intestine is commonly hyperæmic, the spleen enlarged, the liver normal. The bacillus is found in the blood in rather small numbers, and may always be obtained in cultures from the blood or the parenchyma of the liver or spleen.

No. 4. Bacillus cavicida Havaniensis. (My bacillus *x x* Havana, 1889.)

This bacillus was obtained from the contents of the intestine of a yellow fever cadaver in Havana, in 1889 (Autopsy No. 24), through inoculated guinea-pigs, as follows:

June 13, 12 m.—Injected subcutaneously into guinea-pig No. 102, 3 minims of viscid mucus from intestine of Case 24; collected at 9 a. m.; not black. The animal died at 8.30 a. m., June 14; considerable collection of bloody serum in subcutaneous connective tissue containing a motile bacillus. June 14, 10 a. m., injected subcutaneously,

into guinea-pig 105, 3 minims of bloody serum from cellular tissue of guinea-pig 102. June 15, 6 a. m., the animal is very feeble, lying upon its side, respiration rapid. Died at 7.30 a. m.; autopsy at once. No subcutaneous œdema, liver pale, abdominal viscera normal in appearance; bacilli in smear-preparation from liver; a few in preparation from blood of heart. Pure culture of motile bacillus from blood of heart; slide 1241.

This is an actively *motile, non-liquefying bacillus.*

It is a *facultative anaërobic.* In gelatine stick-cultures the growth upon the surface is very scanty and thin, not extending far from the point of puncture; along the line of puncture are developed small translucent, pearl-like, spherical colonies, which later become opaque and sometimes granular.

In gelatine roll-tubes, at the end of 24 hours at 22° C., the deep colonies are very small spheres of a pale straw-color, later they become opaque light-brown spheres, or may have a dark central mass surrounded by a transparent zone. The superficial colonies at the end of 5 days are small translucent masses of a pale straw color towards the center, with thin and irregular margins; sometimes with a central light-brown nucleus; at end of 10 days the deep colonies are still quite small, of a brown color, and opaque.

In glycerine-agar roll-tubes at end of 24 hours the deep colonies are in the form of a biconvex lens, and appear spherical when viewed in face and biconvex when seen from the side; they have a straw color by transmitted light, and are bluish white by reflected light; the superficial colonies are translucent with a bluish-white luster.

On potato, at 22° C., at the end of 48 hours there is a thin dirty yellow growth of limited extent; at the end of 10 days there is a thin gamboge-yellow layer and little masses of the same color; the growth is quite thin, with irregular outlines, and is confined to the vicinity of the impfstrich.

Grows in nutrient agar containing 0.2 per cent. of hydrochloric acid (1:500) Thermal-death-point 130° F. (about 55° C.).

Grows in agua coco without forming gas, and causes the liquid to become slightly translucent, not milky.

Not killed by 30 minutes exposure to a temperature of 10° F.

In its *morphology* this bacillus closely resembles the colon bacillus.

It does not correspond with the descriptions of bacillus cavicida (Brieger's bacillus). This, according to Flügge and Eisenberg, forms very characteristic colonies. "Sehr charakteristic, in form sehr schön gruppierter, weisslicher, konzentrischer ringe, die änlich angeordnet sind, wie die Schuppen auf dem Rücken einer Schildkröte" (Eisenberg).

I have not seen anything answering to this description in the colonies of the bacillus under consideration. In order to make the comparison with Brieger's bacillus, I obtained from Dr. A. C. Abbott a culture of this bacillus from the stock preserved in Professor Welch's laboratory in Baltimore. This also did not present the characteristic colonies

above described, nor did it kill guinea-pigs. Since my return to Baltimore I have again obtained a culture from the same source, and again have been unsuccessful in killing guinea-pigs with it. A careful comparison side by side with the bacterium coli commune shows that in its characters of growth and in its morphology this bacillus of Brieger is identical with the bacillus of Escherich. Whether the cultures preserved in German laboratories preserve their pathogenic power I am unable to say.

The cultures of my bacillus cavicida Havaniensis which I brought to Baltimore with me, upon being replanted after an interval of 3 months, failed to grow. I have therefore lost this interesting bacillus from my collection.

It is very pathogenic for guinea-pigs, not so pathogenic for rabbits. This is shown by the following experiments:

Havana, June 16, 1889, 12:30 *p. m.*—Injected subcutaneously into guinea-pig 109, 3 minims of a culture in agua coco from heart of guinea-pig 105 (see above). Animal found dead next morning at 6 o'clock. Very slight subcutaneous œdema near point of inoculation. Liver light in color, contains a few bacilli; stomach hyperæmic.

June 17, 10 *a. m.*—Injected subcutaneously into guinea-pig 110, 4 drops of a culture in agua coco from heart of guinea-pig No. 105. Animal died at 10 p. m. same day. Considerable subcutaneous œdema containing bacillus *x x*. Bacilli in liver not numerous.

June 17, 10 *a. m.*—Injected subcutaneously into guinea-pig 110 4 four drops of a culture in agua coco from heart of guinea-pig No. 105. Animal died at 10 p. m. same day. Considerable subcutaneous œdema containing bacillus *x x*. Bacilli in liver not numerous.

June 17, 10 *a. m.*—Injected into cavity of abdomen of guinea-pig No. 112 ½ cubic centimetre culture bacillus *x x* in agua coco. Died June 18 at 12 m.

June 18, 7 *a. m.*—Injected subcutaneously into guinea-pig 113, 1 drop of bloody terum from cellular tissue of guinea-pig 110 (see above). Died at 9 p. m., next day. But little subcutaneous effusion; liver light in color, contains much fat, spleen large.

June 19, 2:30 *p. m.*—Injected subcutaneously into guinea-pig 114, 1 drop of fluid from anærobic culture in glycerin agar of bacillus *xx*. June 22, the animal has been very sick but now appears better. June 24, appears well. Died June 29, culsure from blood of heart, negative.

June 23, 10 *a. m.*—Injected subcutaneously into guinea-pig 118, one-half cubic centimetre culture of bacillus *xx* in agua coco (third culture from heart of guinea-pig 105). Animal died at 9 p. m. the same day.

July 17, 8 *a. m.*—Injected subcutaneously into guinea-pig 155, one-half cubic centimetre culture of bacillus *xx* in agua coco, from agar stick culture two weeks old. Found dead at 6 a. m. next morning. Extensive collection of bloody serum in walls of abdomen.

EXPERIMENTS ON RABBITS.

Havana, June 20, 1889.—4 *p. m.*—Injected subcutaneously into rabbit No. 114, weight 900 grams, 1 cubic centimetre culture bacillus *xx* in agua coco. June 22, remains well but has a collection of bloody serum in walls of abdomen from which a pure culture of bacillus *xx* was obtained. Died in convulsions at 6 a. m. July 23.

June 23, 1889, 10 *a. m.*—Injected subcutaneously into rabbit 115, one-half cubic centimetre culture of bacillus *xx* in agua coco. Dead at 6 a. m. July 25. Has had a profuse, watery diarrhœa; no subcutaneous œdema.

June 24, 4 *p. m.*—Injected subcutaneously into rabbit No. 117, 1 cubic centimetre culture bacillus *xx* in agua coco. June 25, 6 a. m., declines food; temperature in rectum 106° F. This animal recovered.

June 26, 9:30 *a. m.*—Injected into cavity of abdomen of rabbit 118, 1 cubic centimetre culture bacillus *xx* in agua coco. Animal recovered.

July 7, 9:30 *a. m.*—Injected into cavity of abdomen of rabbit 123, 1 cubic centimetre culture of bacillus *xx* in agua coco. Recovered.

No. 5. Bacillus hepaticus fortuitus. (Sternberg.)

Obtained in cultures from bloody serum in connective tissue of guinea-pig 119, inoculated with blood from liver of case 28.

An *aërobic, non-liquefying* bacillus, not observed to be motile, or to form spores.

In gelatine stick cultures does not grow along line of puncture except to a slight extent near surface. On surface a white mass is formed about the point of puncture. See Fig. 8, Pl. VIII.

In gelatine Esmarch roll-tubes forms spherical light brown colonies, homogeneous or finely granular; at end of 4 days deep colonies are lobate, and the superficial are like a mamma in form, with striations radiating from the center, and of a dark brown color.

Upon surface of agar stick culture a soft and rather thin white layer is formed at end of 3 days at 26° C.

Upon the surface of glycerin-agar the development is quite rapid, nearly the entire surface being covered at end of 24 hours with a milk-white growth, in incubating oven at 35° C.

On potato at end of 48 hours a rather dry and thick cream-white growth formed along the impfstrich at end of 48 hours. The potato has a bluish discoloration which afterwards disappears; at end of 2 weeks a rather thin, light brown semi-fluid layer covers the entire surface.

Abundant growth in agua coco at end of 24 hours at room temperature, without formation of gas.

Resembles bacterium coli commune in its morphology, but is differentiated from this bacillus by the fact that it is strictly aërobic, by its colonies in gelatine plate cultures, etc. See Fig. 1, Pl. VIII.

Not pathogenic for rabbits (single experiment in which 1 cubic centimetre of a culture in agua coco was injected into cavity of abdomen).

No. 6. Bacillus intestinus motilis. (Sternberg.)

Obtained in cultures from the contents of the intestine of yellow-fever cadavers in Havana, 1889.

A small, actively *motile, non-liquefying* bacillus of the "colon group." A *facultative anaërobic.* In gelatine stick cultures pale straw-colored colonies along line of puncture to bottom, and a rather thin translucent white layer upon surface. Sometimes a nebulous outgrowth occurs from the line of puncture and tufted outlying colonies are formed throughout the gelatine; at other times, in old cultures, a few feathery tufts sprout out from the line of puncture.

In gelatine roll-tubes, at end of 24 hours at 27° C. the deep colonies are spherical and homogeneous and of a pale straw color; superficial colonies are like little drops of water, of a pale brown color.

Grows in agua coco without forming gas.

On potato the growth is rather thin and of a pale yellow color, not extending far from line of impfstrich.

In its morphology this bacillus resembles the "colon bacillus" of Escherich, but it is distinguished from it by its active movements, its colonies in gelatine roll-tubes, etc. See Fig. 2, Pl. VIII.

Not pathogenic for guinea-pigs; not tested upon other animals.

No. 7. Bacillus cavia fortuitus. (Sternberg.)

Obtained in pure culture from liver of guinea-pig 134, inoculated subcutaneously with 2 minims of material from liver of case 27, preserved 48 hours in an antiseptic wrapping.

An actively *motile*, *non-liquefying* bacillus. *Facultative anaërobic.*

In gelatine stick cultures there is a scanty growth on surface at point of puncture; growth to bottom of stick, where the colonies are spherical, translucent, straw-colored and pearl-like by reflected light. Colonies in gelatine roll-tube at end of three days, small, spherical, light brown in color at first, later opaque, sometimes with an opaque granular central portion surrounded by a transparent zone.

Growth on potato at end of a week in form of small dirty yellow masses.

Does not form gas in agua coco.

The morphology of this bacillus is shown by my photomicrograph. Fig. 3, Pl. VIII.

A culture in agua coco (1 cubic centimetre) injected subcutaneously into guinea-pig 138 gave a negative result. No further experiments made with reference to pathogenic power.

No. 8. Bacillus caniculacida. (Koch.)

I obtained the bacillus of rabbit septicæmia in Havana under the following circumstances: A guinea-pig was inoculated subcutaneously on the 31st of July with 2 minims of material from the liver of case 29, kept 48 hours in an antiseptic wrapping. The animal died at the end of 26 hours and 1 cubic centimetre of a culture in blood serum from its liver was injected on the 6th of August beneath the skin of a rabbit. The animal died at the end of 22 hours, and a culture of the bacillus of rabbit septicæmia was obtained from its liver. Two drops of blood from the heart of the above rabbit injected beneath the skin of another rabbit caused its death at the end of 20 hours. Its blood contained the same bacillus.

On the 10th of August another rabbit was inoculated subcutaneously with 3 minims of material from the liver of case 31. This animal died in convulsions at the end of 48 hours, and the bacillus of rabbit septicæmia was recovered from its liver.

On the 11th of August at 3 p. m. one-half cubic centimetre of a culture of this bacillus in veal broth was injected beneath the skin of another rabbit. This animal died at 9 p. m. the same evening and the bacillus was found in abundance in its blood.

Having in the rabbit a test for the presence of this bacillus, and having found it in two cases, I continued the search for it in some subsequent autopsies as follows:

August 12, 1:30 *p. m.*—Injected beneath the skin of rabbit No. 164, 5 minims of liver pulp from case 32. Result negative.

August 13, 12:30 *p. m.*—Injected subcutaneously into rabbit 169, 4 minims of liver pulp from case 33. Result negative.

August 15, 1:30 *p. m.*—Injected subcutaneously into rabbit 171, 1 cubic centimetre of material, principally blood, from case 35. Result negative.

August 17, 9:30 *a. m.*—Injected subcutaneously into rabbit 176, 4 minims from liver of case 35, kept 48 hours in antiseptic wrapping, material contains bacillus N and other bacilli. Animal found dead at 6 a. m. August 21. Bacillus of rabbit septicæmia not present. A motile bacillus obtained in cultures from liver.

August 19, 7:30.—Injected subcutaneously into rabbit 178, one-half cubic centimetre crushed parenchyma from liver of case 36. Result negative.

August 21, 10:30 *p. m.*—Injected subcutaneously into rabbit 183, 2 minims material from liver of case 37. Result negative.

August 22, 1:45 *p. m.*—Injected subcutaneously into rabbit 184, 1 cubic centimetre blood and crushed parenchyma from liver of case 38. Result negative.

These experiments suffice to show that the presence of this widely distributed pathogenic bacillus in two cases was accidental, and of no significance so far as the etiology of yellow fever is concerned.

No. 9. Bacillus Havaniensis (Sternberg).

This is an *aërobic*, *chromogenic* bacillus (micrococcus?) which I obtained in my cultures from the kidney in a single case, in Havana, in 1888. It is extremely small, as will be seen by reference to my photomicrograph, Fig. 1, Pl. IX, and should perhaps be described as a micrococcus.

It is *not motile*, and, so far as my observations go, does not form spores.

It does not liquefy gelatine, but grows upon the surface of flesh peptone-gelatine as an opaque, brick red or carmine layer, which develops slowly and extends very gradually from the point of inoculation. There is a scanty development near the surface in gelatine stick cultures, but the characteristic color is only formed upon the surface where there is free access of oxygen.

On nutrient agar the growth is slow but continuous, forming at the end of 10 days at the room temperature a heaped-up carmine mass.

Frequently this bacillus fails to grow upon the surface of cooked potato, perhaps because of an acid reaction of the potato. But sometimes it grows as it does on nutrient agar, forming a thick irregular mass of a bright carmine color, as seen in Fig. 1, Pl. XIX. Dr. Kemp, of the Hoagland laboratory, who succeeded in obtaining a culture on potato after I had failed, thinks that it grows more readily on cooked potato which has been kept for some time and has become dry.

In gelatine roll-tubes the colonies are small, spherical, translucent, and of a beautiful blood-red color.

I have a variety of this bacillus in cultivation which has scarcely any

color—a trace of pink only. This is from a pure culture which had the usual deep carmine color and which I kept for a year in a hermatically sealed glass tube. Upon replanting it at the end of this time in nutrient agar it grew, but has since been almost without color. The growth is also less abundant. This bacillus is *not pathogenic* for guinea pigs. I have not tested it upon other animals.

No. 10. Baccillus vacuolosis (Sternberg).

I obtained this bacillus in one case in my cultures from the intestine, in one case from the stomach, and in one case a few colonies were obtained in cultures from the kidneys.

The *morphology* is shown by my photomicrograph, Fig. 3, Pl. IX. It varies considerably in its dimensions, especially in old cultures, in which a variety of involution forms are encountered. The rods are often more or less curved and may grow out into long-jointed filaments. They present the appearance of containing numerous vacuoles in the protoplasm; these are not spores, but this bacillus forms, under certain circumstances, large oval spores. It is sometimes motile, the movements being slowly progressive, with a to and fro movement, as if propelled by a flagellum. It *liquefies gelatine* slowly in cup shape, the liquefied gelatine being quite viscid with a cream-white layer of bacilli on the surface. It does not grow in an acid medium.

In nutrient agar the growth along the line of puncture is scanty; on the surface it forms a cream-white layer and the bacilli grow out into long-jointed filaments.

On potato it forms a thin cream-white layer.

Not pathogenic for rabbits; not tested upon other animals.

No. 11. Bacillus fluorescens liquefaciens.

This bacillus I first encountered in Havana, in 1888, in cultures from the spleen, stomach, and fluid in the peritoneal cavity of case 2. In my researches in Decatur, in the autumn of the same year, I obtained it in a number of cases in my cultures from the feces of yellow-fever patients.

It is a *motile, liquefying* bacillus, which forms a greenish pigment and gives to the liquefied gelatine a fluorescent greenish color (see Fig. 2, Pl. XXI). A thick white deposit, consisting of bacilli, settles to the floor of the liquefied gelatine.

On *potato*, at the end of two weeks, at the room temperature a rather thin pale brown layer covers the greater part of the surface; this has a varnished shining appearance as seen in Fig. 1, Pl. XXI.

This bacillus is about 0.8 μ in diameter and four or five times as long as broad. It is frequently joined in pairs, as seen in my photomicrograph, Fig. 2, Pl. IX.

I have made no experiments with reference to its pathogenic power, having lost my culture of it during my absence in Havana last summer.

No. 12. Bacillus pyocyanus (Gessard).

Obtained from the liver of case 28, Havana, 1889, and from the feces of a case in Decatur, in 1888; a liquefying bacillus which forms a green pigment and appears to be identical with the bacillus pyocyanus.

It is actively motile. Its morphology is shown in my photomicrograph, Fig. 1, Pl. X, and its growth in gelatine by Fig. 2, Pl. IX.

Upon potato a chocolate-colored layer is formed, as shown in Fig. 3, Pl. XIX. The potato sometimes acquires a green color and sometimes does not.

Agar cultures acquire a beautiful fluorescent green color, which penetrates the medium from the surface, where the pigment is formed in presence of oxygen. Old agar cultures acquire an olive-brown color. The growth upon the surface of agar is abundant, of a white color tinted slightly green, and is quite viscid.

A thin mycoderma forms upon the surface of the liquefied gelatine in gelatine cultures, and it is here that the pigment is formed.

Young colonies in gelatine roll-tubes are coarsely granular, light-brown in color, and are sometimes surrounded by a transparent ruffle-like margin, as shown in Fig. 3, Pl. X. Later liquefication occurs, as is shown in Fig. 4 of the same plate.

This bacillus is pathogenic for guinea-pigs and rabbits, as shown by the following experiments:

Baltimore, March 13, 1890, 10 *a. m.*—Injected subcutaneously into guinea-pig 209 one-half cubic centimetre liquefied gelatine-culture of bacillus, 12. Found dead next morning at 8 o'clock; bacillus pyocyanus recovered from blood of heart.

March 10, 9:30 *a. m.*—Injected into cavity of abdomen of rabbit 1 cubic centimetre liquified gelatine-culture of bacillus 12. Animal died without convulsions at 1 o'clock the same day.

March 10, 9:30 *a. m.*—Injected subcutaneously into rabbit 258, weight 2,075 grammes, 1 cubic centimetre liquefied gelatine-culture of bacillus 12. Animal recovered, and on the 22d of March received in the cavity of the abdomen 2 cubic centimetres liquefied gelatine-culture of the same bacillus. Result negative. (Protected by first inoculation.)

March 13, 10 *a. m.*—Injected into cavity of abdomen of rabbit No. 262, weight 720 grammes, one-half cubic centimetre liquefied gelatine-culture of bacillus 12. Animal found dead next morning at 8 o'clock. Bacillus recovered in pure culture from liver.

March 13, 10 *a. m.*—Injected subcutaneously into rabbit No. 263, weight 650 grammes, one-half cubic centimetre liquefied gelatine-culture of bacillus 12. Animal found dead next morning at 8 o'clock. Bacillus recovered in pure culture from liver.

March 15, 9:30 *a. m.*—Injected into cavity of abdomen of rabbit No. 274, weight 505 grammes, 1 cubic centimetre *sterile* culture of bacillus 12. Result negative. March 22, 11 a. m., injected into cavity of abdomen of the same rabbit 2 cubic centimetres liquefied gelatine-culture of bacillus 12. Animal remained in good health. Protection by injection of a sterile culture.

No. 13. Bacillus liquefaciens commune. (My bacillus *o*, Decatur, 1888).

This bacillus was present, in comparatively small numbers, in about half the cases, in my cultures from the feces of yellow fever patients, made at Decatur, Ala., in the autumn of 1888.

It is an actively *motile, liquefying* bacillus.

In its *morphology* it resembles the colon bacillus, being a short rod with rounded ends, three or four times as long as broad. See my photomicrograph, Fig. 2, Pl. x.

In *gelatine* stick cultures liquefaction occurs rapidly in the form of a purse, as is shown in Fig. 6, Pl. x.

Growth occurs at a comparatively low temperature; a culture exposed in an attic room in Baltimore in the month of January showed decided development with liquefaction of the gelatine.

On *potato* the growth at the end of two weeks is of pinkish color, and more or less irregular and corrugated, as shown in Fig. 3, Pl. xxi.

This bacillus grows readily in a gelatine medium containing 0.2 per cent of hydrochloric acid.

The following experiments show that it is not decidedly pathogenic for rabbits:

Baltimore, December 5, 1888.—Injected subcutaneously into rabbit No. 59, weight 872 grammes, 2 cubic centimeters liquefied gelatine-culture bacillus *o*. The animal had some cellulitis at point of inoculation, and an abscess, but recovered.

Baltimore, December 5, 1888.—Injected subcutaneously into rabbit No. 60, weight 545 grammes, one-fourth cubic centimetre liquefied gelatine-culture of bacillus *o*. Some cellulitis at point of inoculation; recovered.

Baltimore, December 12, 1888.—Injected into cavity of abdomen of rabbit 64, weight 715 grammes, 2½ cubic centimeters liquefied gelatine-culture of bacillus *o*. Abscess formed at point of inoculation, but animal recovered.

No. 14. Bacillus subtilis. (Ehrenberg.)

In my autopsy No. 12, made at Decatur, Ala., in the autumn of 1888, I obtained in my cultures from the stomach and intestine a *motile, liquefying* bacillus, which proved to have all the characters of the widely distributed species known under the above name. In Havana, in 1889, I obtained the same bacillus in cultures from the surface of the body of yellow-fever patients in the Civil Hospital. This is a large bacillus, which grows out into long-jointed filaments and forms large oval spores. It liquefies gelatine quite rapidly in the form of a purse. On potato, at the end of 10 days a rather dry, dirty-white layer covers the entire surface; this is made up of free spores and spore-bearing filaments.

No. 15. Bacillus subtilis similis (Sternberg).

This is a *liquefying, motile* bacillus, which forms large *oval* spores, and resembles bacillus subtilis. I obtained it in cultures from the liver of case 22, Havana, 1889. It is a *facultative anaërobic.*

In gelatine roll-tubes the young colonies at the end of 36 hours, at room temperature, are spherical, finely granular, and pearl-like by reflected light. The superficial colonies at the same time have commenced to liquefy and have a granular white mass at the center surrounded by liquefied gelatine. See Fig. 2, Pl. xi.

The morphology is shown by my photomicrograph, Fig. 1, Pl. xi, in which the oval spores are seen in the interior of some of the rods. This bacillus does not liquefy gelatine as rapidly as bacillus subtilis. In a

gelatine stick culture at 70° F. at the end of 10 days the upper half of the gelatine was liquefied and small pearl-like colonies were scattered along the line of puncture below. On the floor of the liquefied gelatine was a floculent white deposit and a thin mycoderma on the surface. The bacilli in recent cultures have a slow to and fro progressive movement, as if propelled by a flagellum.

On potato, at 86° F., a dry, yellowish-white layer the size of a dime was formed at the end of 48 hours. In this culture the bacillus had grown out into long jointed filaments containing spores. In Baltimore at a later date the growth on potato was a dry, white growth of limited extent at the end of 5 days.

On the surface of nutrient agar a thick cream-white layer is formed at the end of 4 or 5 days at the room temperature. Along the upper portion of the line of puncture there is a branching growth. In an agar culture of 12 days the growth upon the surface contained but few spores, and variously contorted involution forms of the bacillus were present.

I have made but a single experiment with reference to the pathogenic power of this bacillus, as follows:

Baltimore, March 1, 1890.—Injected into the cavity of the abdomen of rabbit No. 239, weight 1,400 grammes, one-half a cubic centimetre, liquefied gelatine-culture of bacillus 15. March 10, weight 1,250 grammes; animal in apparent good health, but has had a large ulcer at the point of injection.

No. 16. Bacillus intestinus liquefaciens (Sternberg).

Obtained from the intestine of case 27, Havana, 1889.

This is a *motile, liquefying* bacillus.

In gelatine stick-cultures the upper portion of the gelatine is liquefied, and pale straw-colored colonies are scattered along the line of puncture below.

Upon *potato* the growth is rather thin and of a pale yellow color.

In its *morphology*, this bacillus presents nothing characteristic; it probably belongs to the "proteus" group. The rods are about 0.8μ in diameter, and two or three times as long as broad. See Fig. 4, Pl. XI.

No. 17. Bacillus filiformis (Sternberg).

Obtained in anaërobic cultures in glycerine-agar from liver of case 36, Havana, 1889.

A *facultative anaërobic.*

No *motion* observed; does not form spores.

Colonies in anaërobic glycerine-agar tube, spherical or irregular in outline, straw color or pale brown, white and opaque by reflected light; superficial colonies are thin and translucent, and have a bluish luster by reflected light; later they appear as cream-like, irregular masses.

No growth in agua coco; scanty, milk-white growth on surface of *nutrient agar* and opaque, branching growth along line of puncture.

No growth upon *potato*.

Scanty growth in *gelatine* stick-culture along line of puncture, none on surface, Fig. 6, Pl. XIII.

Grows in neutral bouillon, causing a slight opalescence, and later a scanty white sediment.

In my anaërobic cultures made in Havana this bacillus was shorter and thicker than it appears in my photomicrograph, Fig. 1, Pl. XIII. In old agar-cultures the bacilli are very much attenuated and appear as long homogeneous filaments of various dimensions.

Not pathogenic for rabbits or guinea-pigs.

No. 18. Bacillus cadaveris (Sternberg.) (Bacillus N, Havana, 1889.)

This is a large *anaërobic* bacillus, which was the most constant and abundant microörganism found in pieces of liver and kidney kept for 48 hours in an antiseptic wrapping. It was also present, in two cases, in smear preparations made from fresh liver tissue. Since my return from Havana I have found it in pieces of liver preserved in the same way from my comparative autopsies.

Fig. 1, Pl. XII, is a photomicrograph from a smear preparation made from the liver of Case 18 (Havana, 1889), preserved for 48 hours in an antiseptic wrapping. Other bacilli are associated with the large anærobic bacillus, but this is the most conspicuous and most abundant. Invariably the tissue containing it has a very acid reaction; it is rather soft but preserves its fresh appearance and has not a putrefactive odor.

This bacillus is usually about twice as long as broad, but may grow out into rather long threads.

The ends of the rods are square or slightly rounded. It is motionless and so far as my observations go does not form spores. In my photomicrographs it is associated with a small coccus in pairs, which I isolated in pure cultures and have studied separately (see p. 218).

Bacillus cadaveris is a strict a naërobic and is difficult to cultivate. I have succeeded best with nutrient agar containing 5 per cent. of glycerine, removing the oxygen thoroughly by passing a stream of hydrogen through the liquefied medium. The colonies in a glycerine agar roll-tube (containing hydrogen and hermetically sealed) are opaque, irregular in outline, granular, and of a white color by reflected light. The culture medium acquires an acid reaction as a result of the development of the bacillus.

An account has already been given of the pathogenic power of liver tissue containing this bacillus (see p. 127). My experiments with pure cultures are given below:

Havana, May 19, 1889.—Injected into cavity of abdomen of rabbit 106 bacillus N from culture in glycerine agar, suspended in 1 cubic centimetre of veal broth. Result, negative.

Havana, May 16, 1889, 10 *a. m.*—Injected subcutaneously into guinea-pig No. 44, 3 minims of liquid from agar-culture of bacillus N. The animal was found dead the next morning at 6 o'clock. Extensive subcutaneous œdema containing bacillus N.

Havana, May 17, 1889, 3 *p. m.*—Injected subcutaneously into guinea-pig 48, 3 minims of liquid from agar-culture of bacillus N. Animal found dead morning of May 21. No subcutaneous œdema; no bacilli in blood.

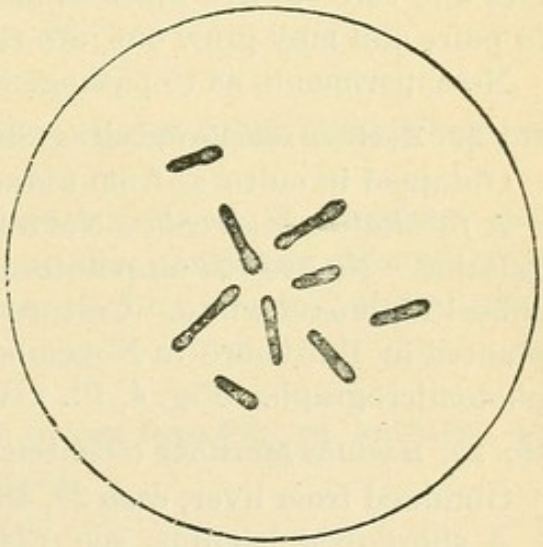
Havana, June 12, 1889, 9:30 *a. m.*—Injected subcutaneously into guinea-pig 97, 5 minims anaërobic culture in glycerine-agar of bacillus N. Animal died at 10 p. m., June 14. No local œdema; abdominal viscera normal in appearance; no microörganisms in blood.

Havana, June 12, 1889, 9:30 *a. m.*—Injected subcutaneously into guinea-pig 98, 10 minims anaërobic culture bacillus N in glycerine-agar. Result, negative.

I regret that these experiments were not extended, inasmuch as they give a somewhat contradictory result. They show, however, that pure cultures of this bacillus are not as pathogenic for guinea-pigs as is the material from yellow fever livers kept in an antiseptic wrapping for 48 hours in which bacillus N is associated with other microörganisms. As none of the microörganisms isolated from such material have shown a virulence correspouding with that of the material itself, I am disposed to think that the local inflammatory œdema which results, especially in guinea-pigs, from injecting it beneath the skin depends upon the presence of the toxic ptomaines present, in connection with the microörganisms which produce these ptomaines.

No. 19. Bacillus cadaveris grandis (Sternberg).

This is a large *anaërobic* bacillus, which was occasionally present in the fragments of liver, etc., kept for 48 hours in an antiseptic wrapping. I have encountered it still more frequently in liver tissue preserved in the same way from my comparative autopsies. I have not obtained it in my cultures. The morphology is shown in Fig. 22, in which the amplification is about 1,000 diameters.



The rods have a rounded extremity and usually contain a large oval spore at one end; sometimes there is a spore at each end.

No. 20. Clostridium cadaveris (Sternberg). (Bacillus T, Havana, 1889.)

This is a *facultative anaërobic, non-motile* bacillus, which I obtained in anaërobic cultures in glycerine-agar associated with Bacillus cadaverinus, from a fragment of liver preserved in an antiseptic wrapping.

It is a clostridium, forming large oval spores in the center of the rods, but these spores are only developed in free contact with oxygen. In agar stick-cultures they are formed upon the surface, while along the line of puncture the rods are longer and may grow out into long filaments as seen in Fig. 4, Pl. XII.

The rods are about 1μ in diameter and 5 to 20 or more in length.

In gelatine stick-cultures the growth upon the surface is very scanty

and thin, of a cream white color; at the bottom of the line of puncture large, transluscent, straw-colored colonies are formed.

In agar stick-cultures the growth along the line of puncture is opaque and upon the surface forms a thin whitish layer.

In bouillon cultures long motionless filaments are formed and the bouillon is slightly clouded.

But a single experiment has been made to test the pathogenic power of this bacillus. One cubic centimetre of a bouillon culture injected beneath the skin of a rabbit gave a negative result.

No. 21. Bacillus anaërobicus liquefaciens (Sternberg).

Obtained in anaërobic cultures from the contents of the intestine. Case 15 (Havana, 1889).

A strictly *anaërobic, non-motile* bacillus.

In anaërobic gelatine roll-tubes it forms granular white colonies, surrounded by liquefied gelatine. No growth in aërobic culture in flesh-peptone-gelatine.

In a long stick culture in nutrient agar it grows along the line of puncture except near the surface.

A retained culture in agar, of May 15, failed to grow when replanted, in Baltimore, in November.

In its morphology this bacillus is a slender rod about 0.6μ in diameter and three to five times as long as broad; the rods are often joined in pairs and may grow out into threads. It forms spores.

No experiments as to pathogenic power.

No. 22. Bacillus renalis fortuitus (Sternberg.)

Obtained in cultures from kidney. Case 18 (Havana, 1889).

A *facultative anaërobic.*—Not observed to be motile. Does not liquefy gelatine. No growth on potato. Superficial colonies in agar roll-tube, spherical, cream white. Culture of May 16th failed to grow when replanted in Baltimore in November. The morphology is shown by my photomicrograph. Fig. 4, Pl. XIV.

No. 23. Bacillus Martinez (Sternberg).

Obtained from liver, case 29, 48 hours in antiseptic wrapping.

A short oval bacillus, see Fig. 2, Pl. XIII. Not motile; does not liquefy gelatine.

Facultative anaërobic.

Growth to bottom of puncture in stick-culture in glycerine-agar, scanty growth on surface.

In glycerine-agar roll-tube a thin, spreading, white growth. In gelatine stick-culture thin and scanty growth on surface and large, spherical, translucent colonies below. Superficial colonies in gelatine roll-tubes like a mamma, with mosaic markings on surface, see Fig. 4, Pl. XIII. Deep colonies spherical and translucent.

Not pathogenic for rabbits. (Single experiment.)

No. 24. Bacillus luteus commune (Sternberg).

Obtained from liver, case 27, 48 hours in antiseptic wrapping. A single colony, probably accidental.

Aërobic; not motile; does not liquefy. Upon the surface of agar cultures a dry yellow mass. Deep colonies in gelatine roll-tubes irregular in outline, granular and yellow in color; superficial colonies have an opaque yellow center and a thin, irregular, translucent margin.

For morphology, see photomicrograph, Fig. 5, Pl. XV.

No. 25. Bacillus L, Havana, 1889.

Obtained in gelatine cultures from feces of yellow-fever case in Havana, 1889.

An actively *motile, liquefying* bacillus. In gelatine stick cultures at end of 4 days at 28° C. the gelatine is liquefied above and is milky in appearance; at end of 9 days the liquefied gelatine is clouded throughout, has a greenish tint near surface, and a heavy white deposit below.

On potato at end of 5 days a pinkish white growth, rather thick, with irregular margins; contains gas bubbles.

In nutrient agar a milk-white growth on surface, and rather scanty, opaque growth along line of puncture.

For morphology, see Fig. 4, Pl. XV.

No. 26. Bacillus C, Havana, 1889.

Obtained in gelatine cultures from kidney, case 18. An actively motile, liquefying bacillus; see Fig. 3, Pl. XV.

No. 27. Bacillus K, Havana, 1889.

Obtained in culture from surface of the body of case in civil hospital, Havana.

A *motile, liquefying* bacillus. Liquefies gelatine in cup-shape near surface; growth to bottom of line of puncture consisting of small, translucent colonies; liquefied gelatine very slightly clouded, almost transparent.

No growth on potato.

In nutrient agar scanty yellowish-brown growth on surface, and branching growth along line of puncture.

For morphology, see Fig. 2, Pl. XV.

No. 28. Bacillus Havaniensis liquefaciens. (Bac. I.)

Obtained in cultures from the surface of the body of patients in the civil hospital, Havana, 1889.

A *motile, liquefying* bacillus, with round ends; grows out into long threads. No formation of spores observed.

In gelatine stick-cultures liquefaction occurs all along the line of puncture, as shown in Fig. 8, Pl. XIV. The liquefied gelatine at end of 4 days, at 22° C., is slightly clouded throughout; in old cultures the liquefied gelatine is quite transparent, and there is a slight flocculent deposit at the bottom of the tube.

On surface of agar stick-culture at end of two weeks a thin, pale-brown layer.

Colonies in gelatine roll-tubes at end of 24 hours, at 22° C., spherical, with a milky opacity, and having a transparent marginal zone, as seen in Fig. 7, Pl. XIV.

Under the microscope the colonies are seen to be finely granular. At the end of 24 hours liquefaction commences.

No growth on potato.

Not pathogenic for rabbits.

For morphology, see Fig. 1, Pl. XIV.

BACILLI OBTAINED IN COMPARATIVE EXPERIMENTS.

No. 29. Bacillus A, Havana, 1889.

Obtained from contents of sewer at its exit, near the margin of the bay.

A *motile*, *liquefying*, *aërobic* bacillus; forms large oval spores.

Liquefies gelatine slowly; a rather thick mycoderma forms on surface of liquefied gelatine.

Milk-white growth on surface of nutrient agar.

In agar roll-tubes a thin spreading growth with irregular margins.

Semi-fluid growth upon potato, with shining surface, as if varnished; growth very rapid; at end of 24 hours, at 27° C., covers entire surface; later a membranous film wrinkled and raised in ridges at center.

For morphology, see Fig. 3, Pl. XIV.

No. 30. Bacillus E, Havana, 1889.

Obtained from feces of healthy individual.

A *motionless*, *liquefying* bacillus.

Forms large oval spores.

Dry and rather thick white mass upon surface of agar stick-culture.

Rapid growth on potato, forming a white, viscid, spongy, or yeast-like mass. For morphology, see Fig. 2, Pl. XIV.

No. 31. Bacillus U, Havana, 1889.

Accidental colony from the air.

Dirty-white growth on potato.

Not studied further. See Fig. 5, Pl. XIV.

No. 32. Bacillus Y, Havana, 1889.

Accidental colony from the air. A large torula-like bacillus. Liquefies gelatine; the liquefied gelatine is very viscid. (See Fig. 6, Pl. XIV.)

No. 33. Bacillus gracilis (Sternberg). (Bacillus 17, 1890.)

Obtained from liver kept 48 hours in antiseptic wrapping, comparative autopsy No. 11.

A *non-motile*, *non-liquefying* bacillus in long chains composed of short oval elements. (See Fig. 1, Pl. XVI.)

In gelatine stick-culture at 22° C., at end of 5 days a rather thick white mass at point of puncture, covering one-third of the surface, closely crowded, opaque colonies at bottom of line of puncture and slender branching outgrowth above.

In gelatine roll-tubes the deep colonies are opaque and spherical; superficial colonies circular, or slightly irregular in outline, white in color, and opaque or slightly transluscent. (See Figs. 3 and 5, Pl. XVI.)

In nutrient agar, at end of 5 days, at 22° C., milk-white growth on surface; opaque growth to bottom of line of puncture.

On potato, at end of 5 days, at 22° C., rather thick cream-white growth with irregular margins along line of impfstrich.

Cultures in bouillon have a milky opacity and a very disagreeable odor.

Grows in *agua coco* without formation of gas.

No formation of spores observed.

This bacillus is pathogenic for rabbits when injected into the cavity of the abdomen, as is shown by the following experiments:

Baltimore, March 14, 1890, 9:30 *a. m.*—Injected into cavity of abdomen of rabbit No. 268, one-half cubic centimetre bouillon culture of bacillus 17. Animal found dead next morning. Liver and spleen normal, kidneys intensely hperæmic. No bacilli seen in smear preparation from liver and kidneys. Bacillus 17 recovered in culture from blood of heart.

Baltimore, March 15, 9 *a. m.*—Injected into cavity of abdomen of rabbit No. 272, weight 580 grammes, 1 cubic centimetre bouillon culture bacillus 17. Very sick at 12:30, died at 3:30. A few bacilli in chains in preparation from blood of heart. Bacillus 17 recovered in cultures from blood of heart.

Baltimore, March 15, 9 *a. m.*—Injected into cavity of abdomen of rabbit No. 273, weight 1,430 grammes, 1 cubic centimetre bouillon culture of bacillus 17. Animal died at 12:30 same day.

Baltimore, March 31.—Injected into cavity of abdomen of rabbit No. 283, weight 1,220 grammes, 1 cubic centimetre bouillon culture bacillus 17. Result negative.

Baltimore, April 4.—Injected into cavity of abdomen of rabbit 284, weight 1,560 grammes, 1 cubic centimetre bouillon culture bacillus 17. Result negative.

SUBCUTANEOUS INJECTIONS DO NOT KILL RABBITS.

Baltimore, March 17.—Injected subcutaneously into rabbit No. 276, weight 276 grammes, 1 cubic centimetre bouillon culture of bacillus 17. Result negative.

Baltimore, March 31.—Injected subcutaneously into rabbit No. 285, weight 910 grammes, 1½ cubic centimetres bouillon culture of bacillus 17. Result negative.

Baltimore, March 31.—Injected subcutaneously into rabbit No. 286, weight 1,280 grammes, 2 cubic centimetres culture in bouillon. Result negative.

STERILIZED CULTURES NOT PATHOGENIC FOR RABBITS.

Baltimore, April 1.—Injected into cavity of abdomen of rabbit No. 287, weight 1,400 grammes, 8 cubic centimetres sterilized culture (at 160° F.) of bacillus 17. Result negative.

Baltimore, March 18.—Injected into cavity of abdomen of rabbit No. 280, weight 940 grammes, 5 cubic centimetres sterilized culture (at 160° F.) of bacillus 17. Result negative.

NOT PATHOGENIC FOR GUINEA-PIGS.

Baltimore, December 5.—Injected subcutaneously into guinea-pig No. 184, 1 cubic centimetre culture of bacillus 17 in agua coco. Result negative.

Baltimore, March 17.—Injected subcutaneously into guinea-pig 210, 3 minims bouillon culture bacillus 17. Result negative. March 22, injected into cavity of the abdomen of same animal 1½ cubic centimetres bouillon culture bacillus 17. Result negative.

Baltimore, March 17.—Injected into cavity of abdomen of guinea-pig 211, 1 cubic centimetre bouillon culture bacillus 17. Result negative.

No. 34. Bacillus coli similis (Sternberg). (Bacillus 24, 1890.)

Obtained from liver kept in antiseptic wrapping, comparative autopsy No. 11.

A *non-motile, non-liquefying* bacillus, resembling the Bacterium coli commune of Escherich, but differing from it in the characters of its colonies in gelatine roll-tubes, in its growth on potato, etc. (See Figs. 4 and 7, Pl. XVI.)

Growth in flesh peptone gelatine as shown in Fig. 6, Pl. XVI.

In gelatine roll-tubes the superficial colonies are homogeneous and translucent at the end of two days; the deep colonies spherical and pale brown in color. Later the deep colonies become opaque, and the superficial colonies are quite thin and have a pale brown color.

On potato there is at 22° C. a thick dirty-white or light-brown growth along the impfstrich.

Not pathogenic for rabbits or guinea-pigs (single experiment on each animal).

No. 35. Bacillus B, Havana, 1889.

Obtained in anaërobic culture from liver of case of heart disease, kept 48 hours in antiseptic wrapping. Grows at bottom of long agar stick.

No growth in gelatine stick-culture. (See Fig. 1, Pl. XV.)

MICROCOCCI.

No. 1. Staphylococcus pyogenes aureus.

Obtained in cultures from yellow-fever liver kept for 48 hours in an antiseptic wrapping (case 8, Havana, 1888); also from stomach (case 11, Decatur, 1888).

No. 2. Streptococcus cadaveris (Sternberg). (Streptococcus pyogenes?)

Obtained from liver of case 14 (Hav., 1889).

A *facultative anaërobic;* does not liquefy gelatine; forms long chains of spherical or slightly oval elements. The cocci when in process of division resemble a short bacillus with stained ends, and a chain of such dividing cocci may be mistaken for a chain of oval bacilli. Indeed, I at first considered this a bacillus resembling that of Babes, and designated it bacillus *o* in the list of microörganisms isolated by me in 1889.

No growth on surface of gelatine stick-cultures; opaque colonies along the line of puncture larger and more opaque than in similar cul-

tures of streptococcus pyogenes made at the same time. Later the isolated colonies at bottom of line of puncture are irregular in outline and granular.

Thin translucent growth on surface of agar culture in Baltimore. In Havana the growth was more abundant, forming a white mass about the point of puncture. Grows well in an acid medium (1:500 of hydrochloric acid). In bouillon and agua coco it forms little flocculi made up of chains.

In old agar cultures is very small and not in chains. Culture in veal broth gives long chains and the cocci are much larger. The individual elements may vary greatly in size in the same chain.

Very thin white growth on surface of potato at end of 12 days. Not found to be pathogenic for guinea-pigs or rabbits. For morphology see photomicrograph, Fig. 1, Pl. XVII.

No. 3. Streptococcus Havaniensis (Sternberg).

Obtained in acid and transparent liquid vomited by yellow-fever patient in Military Hospital, Havana, 1889, after having been kept for 24 hours in collecting bulb. (See Fig. 2, Pl. XVII.)

No. 4. Streptococcus liquefaciens (Sternberg). (Streptococcus coli gracilis of Escherich ?)

Obtained from liver case 42, 48 hours in laboratory; also from liver case 31, and intestine case 15; also in comparative autopsies in Baltimore.

A liquefying coccus which forms short chains, possibly the Babes microbe.

In gelatine stick cultures the gelatine is entirely liquefied at the end of a week as seen in Fig. 7, Pl. XVII. The liquefied gelatine is but slightly opalescent and there is a scanty deposit at the bottom of the tube.

In nutrient agar there is a scanty growth at the point of puncture and closely crowded opaque colonies to bottom of the line of puncture.

Thin and limited dry white growth on potato at end of 5 days.

Not pathogenic for guinea-pigs or rabbits.

For morphology see photomicrograph, Fig. 4, Pl. XVII.

No. 5. Micrococcus hepaticus (Sternberg).

Obtained in culture from liver, case 8. kept 48 hours in antiseptic wrapping. A diplococcus. (See Fig. 2, Pl. XVIII.)

Does not liquefy gelatine.

In gelatine roll tubes the superficial colonies are spherical and translucent; deep colonies at first translucent and homogeneous, become in 3 or 4 days more or less lobate, and pale brown in color by transmitted light.

Not pathogenic for rabbits.

No. 6. Micrococcus Finlayensis (Sternberg).

Obtained by Dr. Finlay in cultures from the liver and spleen of a yellow-fever case and sent to me by him in August, 1888.

This is a liquefying staphylococcus, which, like other staphylococci, is sometimes found in groups of four. When sent to me Dr. Finlay supposed it to be identical with the large micrococcus in tetrads which he had previously encountered in connection with yellow-fever cases, my *Micrococcus tetragenus versatillis*. He is now satisfied that it is a different species.

This coccus differs from that of Dr. Freire of Brazil in having a pale yellow color when viewed in mass upon the surface of an agar culture; while that of Freire has a milk-white color. (See Fig. 2, Pl. XX.)

It liquefies gelatine slowly, forming a cup-shaped cavity, which has a very viscid, opaque, pale yellow lining, made up of the cocci.

Not pathogenic for guinea-pigs or rabbits.

For the morphology of this coccus see Fig. 4, Pl. XVIII.

No. 7. Micrococcus versatilis albus.

Accidental colony from the air, Havana, 1889.

A liquefying coccus; often in groups of four; very irregular in grouping and dimensions. Milk-white growth on surface of nutrient agar, opaque irregular growth along line of puncture. Milk-white and later grayish-white rather thick growth on potato. Does not form gas or produce acid reaction in agua coco. (See Fig. 5, Pl. XVIII.)

No. 8. Micrococcus luteus.

Obtained in cultures made from the surface of the body of patients in Civil Hospital, Havana, 1889. Does not liquefy gelatine. Minute, opaque colonies along line of puncture in gelatine stick cultures. Yellow growth upon surface of agar.

Gamboge-yellow growth of limited extent on potato. (See Fig. 3, Pl. XVII.)

Torula gastricus (Sternberg).

In my direct examination of vomited matters and of the contents of the stomach after death I have very frequently encountered cells of a torula, and in several cases I have isolated this in pure cultures. It forms a white mass upon the surface of nutrient agar or gelatine cultures; it does not liquefy gelatine, and in Esmarch roll tubes it forms beautiful stellate colonies. The morphology is shown by my photomicrograph, Fig. 1, Pl. XVIII.

The list of micrörganisms above described by no means includes all of those encountered in my studies. I have notes relating to many others, but not having found time to study their characters fully, so as to differentiate them in a satisfactory manner, I have not thought it worth while to make any further mention of them.

IX.—CONCLUSIONS.

The experimental data recorded in this report show that—

The specific infectious agent in yellow fever has not been demonstrated.

The most approved bacteriological methods fail to demonstrate the constant presence of any particular microörganism in the blood and tissues of yellow-fever cadavers.

The microörganisms which are sometimes obtained in cultures from the blood and tissues are present in comparatively small numbers, and the one most frequently found (*Bacterium coli commune*) is present in the intestine of healthy individuals, and consequently its occasional presence can not have any etiological import.

A few scattered bacilli are present in the liver, and probably in other organs, at the moment of death. This is shown by preserving portions of liver, obtained at a recent autopsy, in an antiseptic wrapping.

At the end of 24 to 48 hours the interior of a piece of liver so preserved contains a large number of bacilli of various species, the most abundant being those heretofore mentioned as occasionally found in fresh liver tissue, viz, *Bacterium coli commune* and *Bacillus cadaveris*.

Blood, urine, and crushed liver tissue obtained from a recent autopsy are not pathogenic, in moderate amounts, for rabbits or guinea-pigs.

Liver tissue preserved in an antiseptic wrapping at a temperature of 28 to 30° C. for 48 hours is very pathogenic for guinea-pigs when injected subcutaneously.

This pathogenic power appears to be due to the microörganisms present and to the toxic products developed as a result of their growth. It is not peculiar to yellow fever, inasmuch as material preserved in the same way at comparative autopsies, in which death resulted from accident or other diseases, has given a similar result.

Having failed to demonstrate the presence of a specific "germ" in the blood and tissues it seems probable that it is to be found in the alimentary canal, as is the case in cholera. But the extended researches made and recorded in the present report show that the contents of the intestine of yellow-fever cases contain a great variety of bacilli and not a nearly pure culture of a single species, as is the case in recent and typical cases of cholera.

Comparatively few liquefying bacilli are found in the feces discharged during life or in the intestinal contents collected soon after death from yellow-fever cadavers.

On the other hand nonliquefying bacilli are very abundant.

The one most constantly and abundantly present is the *Bacterium coli commune* of Escherich.

This is associated with various other baccilli, some of which are strict anaërobics and some facultative anaërobics.

Among the facultative anaërobics is one—my bacillus *x*—which has been isolated by the culture method in a considerable number of cases and may have been present in all. This bacillus has not been encountered in the comparative experiments made. It is very pathogenic for rabbits when injected into the cavity of the abdomen.

It is possible that this bacillus is concerned in the etiology of yellow fever, but no satisfactory evidence that this is the case has been obtained by experiments on the lower animals, and it has not been found in such numbers as to warrant the inference that it is the veritable infectious agent.

All other microörganisms obtained in pure cultures from yellow-fever cadavers appear to be excluded, either by having been identified with known species, or by having been found in comparative researches made outside of the area of yellow-fever prevalence, or by the fact that they have been found only in small numbers and in a limited number of cases.

Finally we remark that many facts relating to the origin and extension of yellow-fever epidemics give support to the inference that the specific infectious agent is present in the dejecta of those suffering from the disease, and that accumulations of fecal matter and of other organic material of animal origin furnish a suitable nidus for the development of the "germ" when climatic conditions are favorable for its growth.

It may be that such a nidus is essential and that the culture media usually employed by bacteriologists do not afford a suitable soil for this particular microbe.

It is also possible that its development depends upon the presence of other microörganisms found in fecal matter, which give rise to chemical products required for the development of this one.

Some of the microörganisms present in the dejecta of yellow-fever patients, as shown by stained smear-preparations, have not developed in the cultures made, either aërobic, or anaërobic. One extremely slender, filiform bacillus, which can only be seen with high powers and which is quite abundant in some of my preparations, has never been obtained in the cultures made, and no doubt there are others in the same category.

That the yellow-fever germ is a strict anaërobic or that it will only grow in a special nidus may be inferred from certain facts relating to the extension of epidemics.

There is no evidence that yellow fever is propagated by contamination of the supply of drinking water, as frequently, and probably usu-

ally, occurs in the case of typhoid fever and cholera. Moreover epidemics extend in a more deliberate manner and are restricted within a more definite area than is the case with cholera and typhoid fever. It is usually at least ten days or two weeks after the arrival of an infected vessel or of a person sick with the disease before cases of local origin occur; and these cases occur in the immediate vicinity of the imported case or infected vessel. When the disease has effected a lodgment the area of infection extends slowly and usually has well-defined boundaries. In towns and cities having a common water supply one portion remains perfectly healthy, while another, and usually the most filthy portion, may be decimated by the scourge.

The experimental evidence recorded and the facts just stated seem to justify the recommendation that the dejecta of yellow-fever patients should be regarded as infectious material and that such material should never be thrown into privy vaults or upon the soil until it has been completely disinfected.

This rule thoroughly enforced, together with an efficient quarantine service and proper attention to the sanitary police of our exposed seaport cities, would, I believe, effectually prevent this pestilential disease from again obtaining a foothold within the limits of the United States.

PLATE I.

Yellow fever blood; first day of sickness; fatal case. Photomicrograph made in Havana in 1879. × 1,500 diameters; enlarged from a photomicrograph of 500 diameters made with Beck's one-fifth inch objective, dry.

[Extracts from report of Havana Commission, 1879.]

"In Havana Dr. Sternberg gave a large share of his time to the microscopic examination and photography of the blood. No chemical examination was attempted. The patients from whom specimens of blood were obtained were mostly soldiers in the military hospital of San Ambrosio. Ninety-eight specimens from 41 undoubted cases of yellow fever were carefully studied and 105 photographic negatives were made, which show satisfactorily everything demonstrable by the microscope.

These photographs were mostly made with a magnifying power of 1,450 diameters, obtained by the use of Zeiss's one-eighteenth inch objective, and Tolles's amplifier. Probably no better lens than the Zeiss one-eighteenth (oil immersion) could have been obtained for this work, and it is doubtful whether any objective has ever been made capable of showing more than is revealed by this magnificent lens. With the power used, organisms much smaller than those described as existing in the blood of charbon or of relapsing fever would be clearly defined.

If there is any organism in the blood of yellow fever demonstrable by the highest powers of the microscope as at present perfected, the photomicrographs taken in Havana should show it. No such organism is shown in any preparation photographed immediately after collection. But in certain specimens, kept under observation in culture cells, hyphomycetous fungi and spherical bacteria made their appearance after an interval of from 1 to 7 days. The appearance of these organisms was, however, exceptional, and, in several specimens taken from the same individual at the same time, it occurred that in one or two a certain fungus made its appearance and in others it did not. This fact shows that the method employed can not be depended upon for the exclusion of atmospheric germs, but does not affect the value of the result in the considerable number of instances in which no development of organisms occurred in culture cells in which blood in a moist state was kept under daily observation for a week or more.

The method employed seemed the only one practicable for obtaining blood from a large number of individuals without inflicting unwarrantable pain and disturbance upon the sick. It was as follows: One of the patient's fingers was carefully washed with a wet towel (wet sometimes with alcohol and at others with water) and a puncture was made just back of the matrix of the nail with a small, triangular-pointed trocar. As quickly as possible a number of thin glass covers were applied to the drop of blood which flowed, and these were then inverted over shallow cells in clean glass slips, being attached usually by a circle of white zinc cement. In dry preparations, which are most suitable for photography, the small drop of blood was spread upon the thin glass cover by means of the end of a glass slip.

The thin glass covers were taken from a bottle of alcohol and cleaned immediately before using, and usually the glass slips were heated shortly before applying the covers, for the purpose of destroying any atmospheric germs which might have lodged upon them. These precautions were not, however, sufficient to prevent the inoculation of certain specimens by germs floating in the atmosphere (Penicillium spores and micrococci); and in nearly every specimen the presence of epithelial cells, and occasionally of a fiber of cotton or linen, gave evidence that under the circumstances such contamination was unavoidable. It is therefore believed that any organism developing in the blood of yellow fever, or of other diseases, collected by the method described or by any similar method, can have no great significance unless it is found to develop as a rule (not occasionally) in the blood of patients suffering from the disease in question, and is proved by comparative tests not to develop in the blood of healthy individuals obtained at the same time and by the same method.

Tried by this test it must be admitted that certain fungi and groups of micrococci, shown in photographs taken from specimens of yellow fever blood collected at the military hospital and preserved in culture cells, can not reasonably be supposed to be peculiar to or to have any casual relation to this disease."

PLATE I.

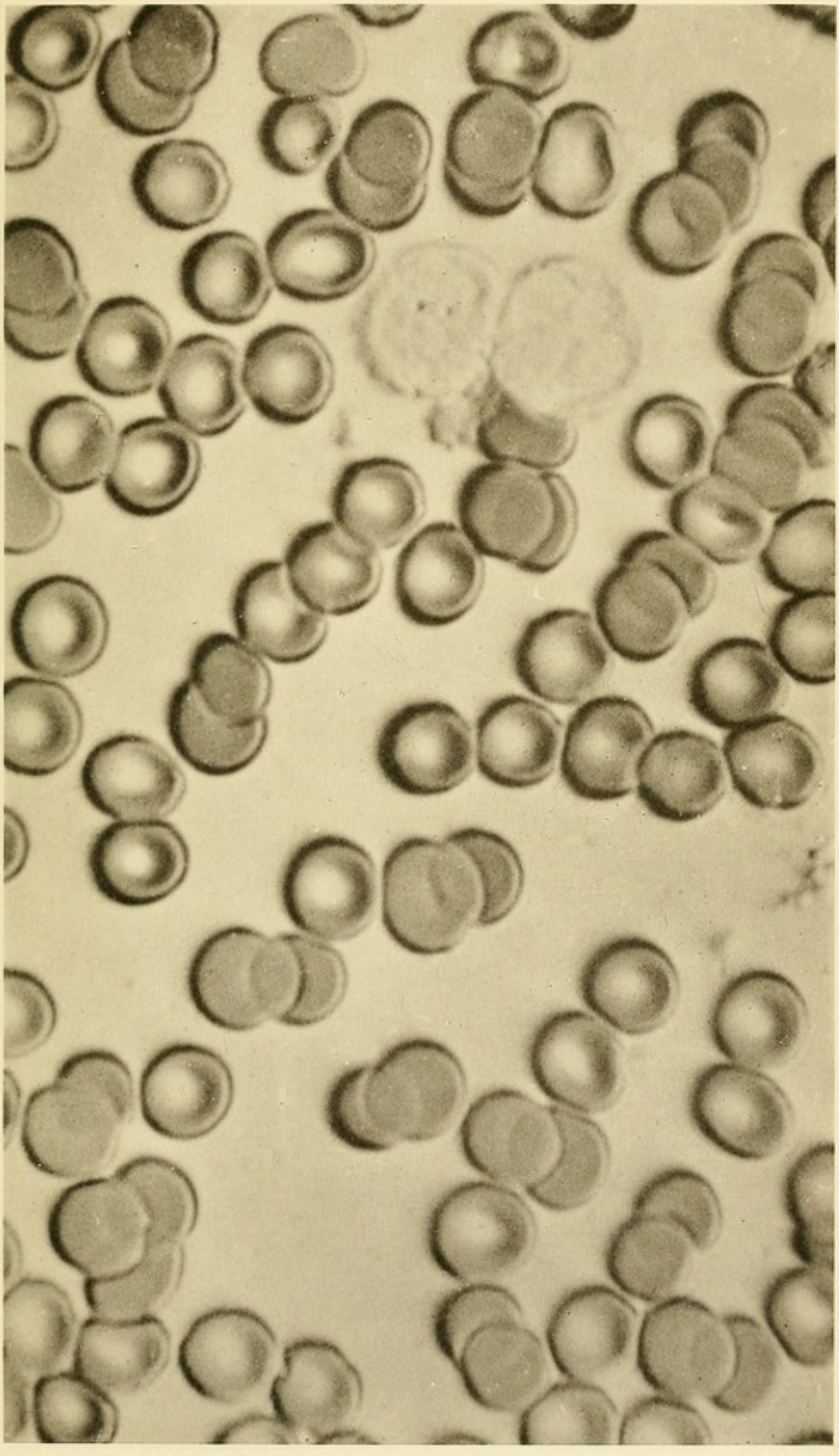


PLATE II.

FIG. 1. Yellow fever blood; fifth day; fatal case. Photomicrograph made in Havana in 1879. × 1,450 diameters by Zeiss's one-eighteenth hom. oil immersion objective and Tolles's amplifier.

FIG. 2. Yellow fever blood; first day; fatal case. Photomicrograph made in Havana in 1879; same amplification as Fig 1.

FIG. 3. Yellow fever blood; fifth day; fatal case. Havana, 1879. Same amplification as Fig. 1.

FIG. 4. Leucocyte in yellow fever blood kept in a culture cell for two days; eighth day of sickness. Fatal case. Havana, 1879. × 650 diameters.

FIG. 5. Leucocyte in yellow fever blood of eighth day; fatal case (same as Fig. 3). Kept in culture cell for two days. × 650 diameters.

[Extract from preliminary report of Havana Yellow Fever Commission to National Board of Health, submitted November 18, 1879.]

"The most important observation made relates to certain granules in the white corpuscles shown in many of the photomicrographs taken. From the manner in which these granules refract light, and for other reasons, they are believed by Dr. Sternberg to be fat, and to represent a fatty degeneration of the leucocytes. The blood of twelve healthy individuals was examined in Havana, for comparison, and in nearly every case an occasional leucocyte was found to contain a few (one or two) granules indistinguishable from those found in the blood of yellow fever; but this was the rare exception, while in severe cases of yellow fever the granules were abundant, and nearly every white corpuscle contained some of them."

REMARK.—In similar preparations (dry) of blood from the finger of yellow fever patients, made in Rio de Janeiro in 1887, the same refractive granules in the leucocytes were encountered. That they are not peculiar to yellow fever is shown by the fact that similar granules were present in the leucocytes, in dry mounts, of blood from the finger of persons suffering from beri-beri in one of the hospitals of Rio de Janeiro.

PLATE II.

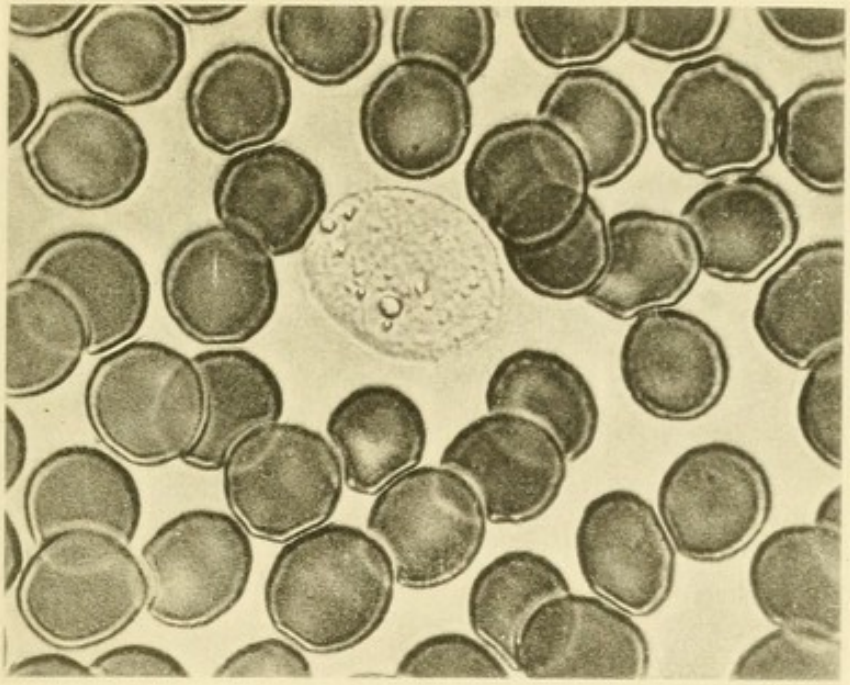


FIG. 1.

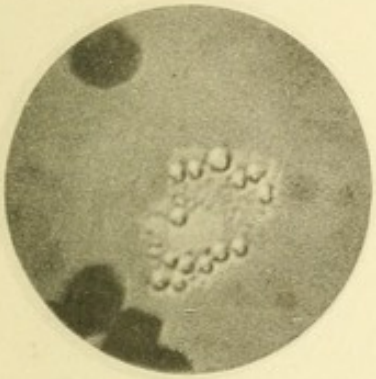


FIG. 4.

FIG. 3.

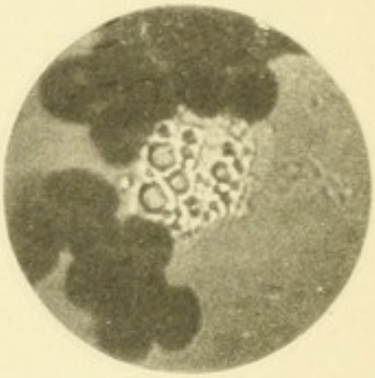


FIG. 5.

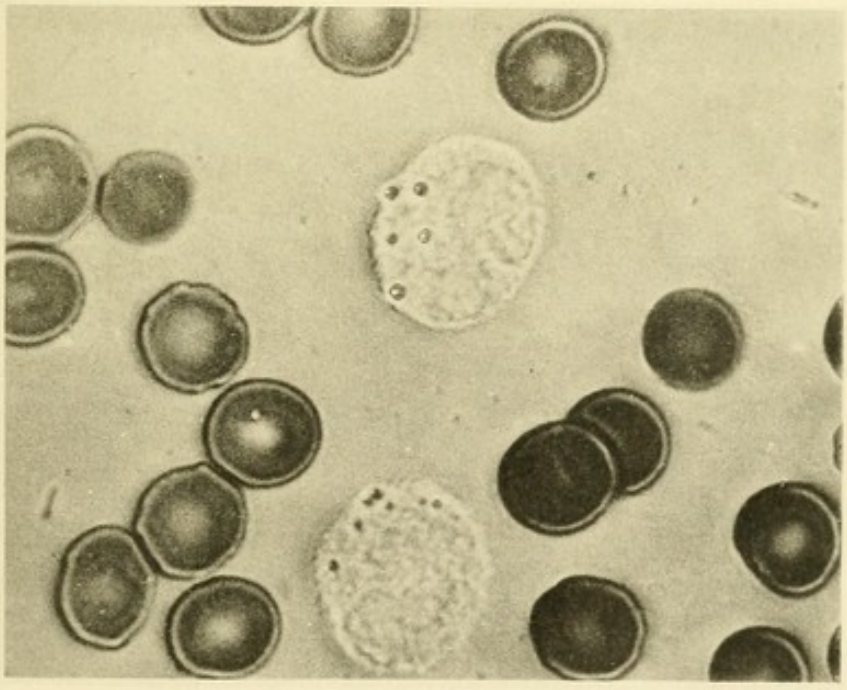


FIG. 2.

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PLATE III.

FIG. 1. Micrococcus of Freire, from the culture given by him to Dr. Sternberg at the time of his visit to Brazil. Fuchsin stain; × 1,000.

FIG. 2. Micrococcus of Freire, from an agar culture; fuchsin stain; × 1,000.

FIG. 3. *Micrococcus tetragenus versatilis* (Sternberg), (tetragenus febris flavæ of Finlay). From a single colony in a gelatine Esmarch roll-tube. Fuchsin stain; × 1,000.

FIG. 4. Bacillus of Carmona, cultivated from yellow fever urine, from a slide mounted in Dr. Carmona's laboratory by Dr. Gaviña, and presented by him to Dr. Sternberg at the time of his visit to Mexico. Fuchsin stain; × 1,000.

FIG. 5. Culture of Freire's micrococcus in flesh-peptone-gelatine at the end of 8 days at 22° C.

FIG. 6. Culture of Freire's micrococcus in flesh-peptone-gelatine at end of 4 days at 22° C.

FIG. 7. *Micrococcus tetragenus versatilis.* Culture in flesh-peptone-gelatine at end of 2 weeks at 22° C.

PLATE III.

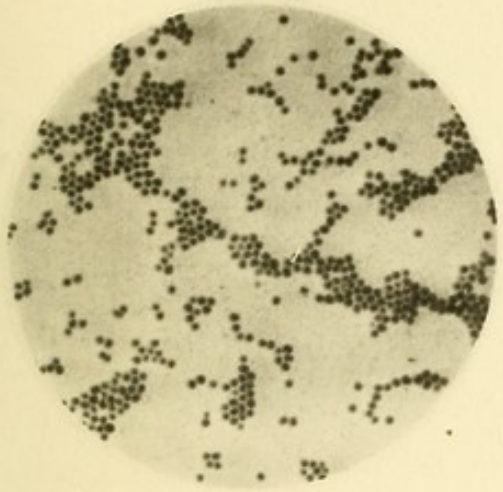
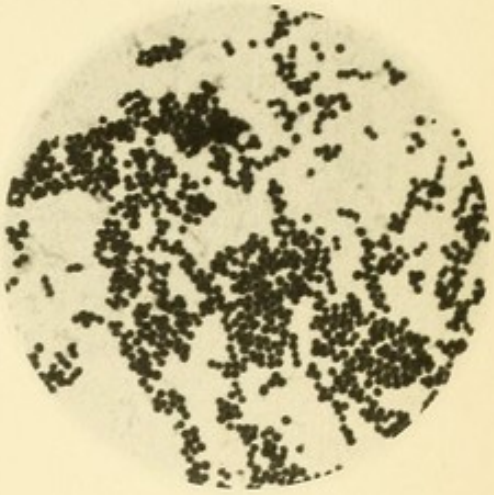


FIG. 1.



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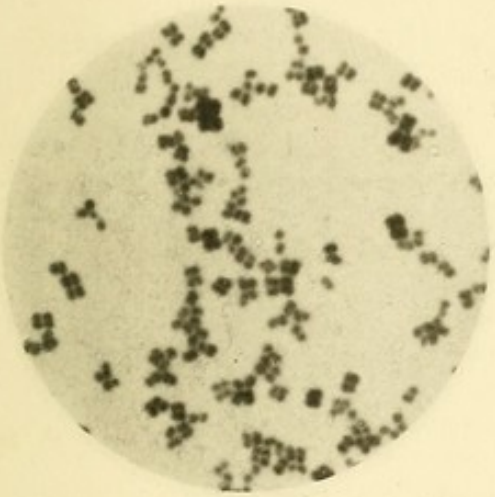

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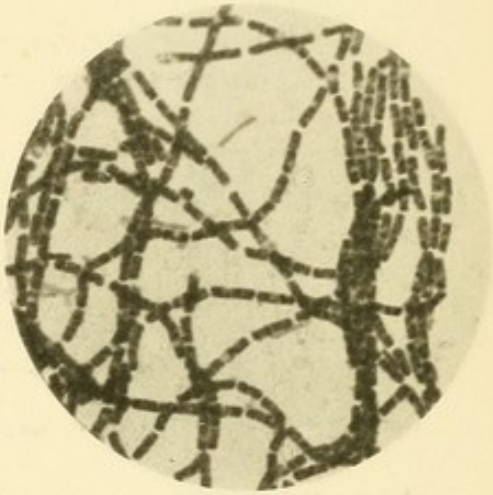

FIG. 4.

FIG. 5.

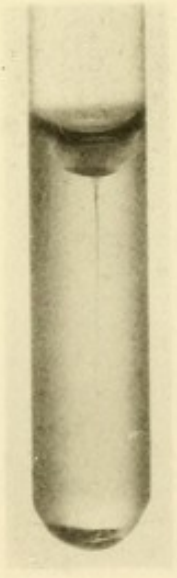

FIG. 6.

FIG. 7.

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PLATE IV.

FIG. 1. *Bacillus lepina lethalis* (Sternberg)—bacillus of Gibier. From surface of agar culture, 12 days old. Fuchsin stain; × 1000.

FIG. 2. *Bacillus lepina lethalis* (Sternberg). From bouillon culture, 24 hours old. Fuchsin stain; ×1000.

FIG. 3. Colonies of Gibier's bacillus in gelatine roll-tube; end of 48 hours at room temperature (about 20° C.); × 5 diameters.

FIG. 4. Single colony of Gibier's bacillus in gelatine roll-tube at end of 3 days at room temporature; liquefaction of gelatine around the colony; × 5 diameters.

FIG. 5. Culture of Gibier's bacillus in flesh-peptone-gelatine; end of 4 days at 22° C.

FIG. 6. Culture of Gibier's bacillus in flesh-peptone-gelatine; end of 5 days at 22° C.

FIG. 7. Culture of Gibier's bacillus in flesh-peptone-gelatine; end of 8 days at 22° C.

PLATE IV.

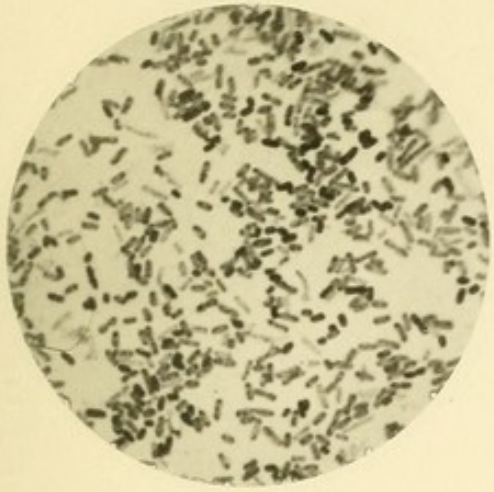
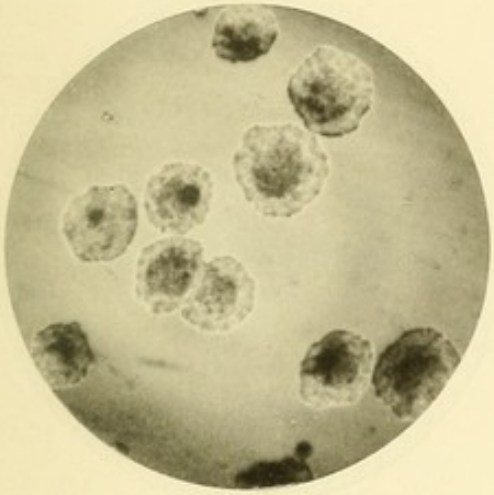


FIG. 1.

FIG. 2.



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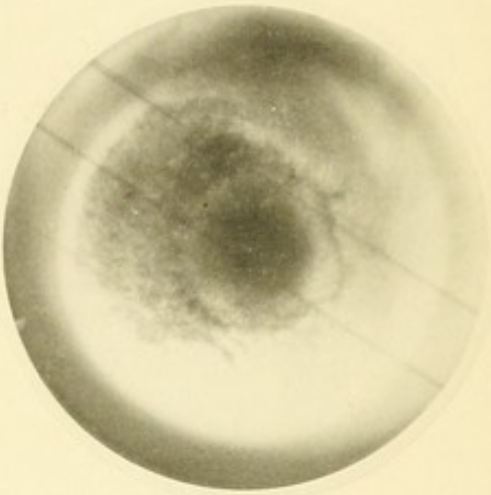

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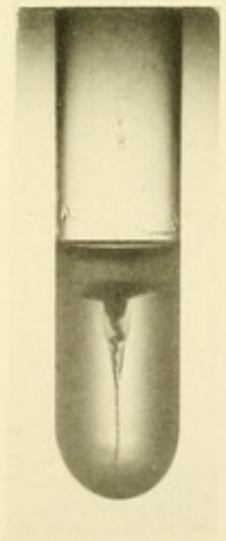

FIG. 5.

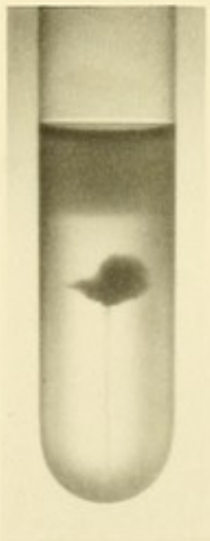

FIG. 6.

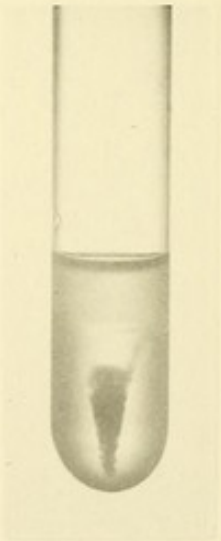

FIG. 7.

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PLATE V.

FIG. 1. Bacillus *a*; "stick-culture" in flesh-peptone-gelatine (20 per cent. of gelatine); from a photograph.

FIG. 2. Portion of same stick-culture shown in Fig. 1, magnified about 4 diameters; from a photograph.

FIG. 3. Bacillus *a*; a gelatine stick-culture obtained from the feathery outgrowths shown in Figs. 1 and 2 (20 per cent. of gelatine).

FIG. 4. Emerich's bacillus cultivated in 20 per cent. gelatine, at 27° C. (80.6° F.)

FIG. 5. Bacterium coli commune of Escherich, cultivated in 20 per cent. gelatine at 27° C. (80·6° F.)

FIG. 6. Bacillus *a*; magnified 1250 diameters. From a photo-micrograph by Dr. Sternberg.

FIG. 7. Colonies of bacillus *a* in flesh-peptone-gelatine; *a* superficial colonies in rosettes; *b* deep colonies, usual form; *c* superficial colonies, usual form.

Fig 1

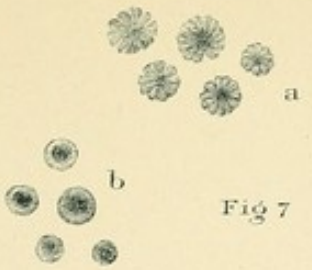

Fig 7

Fig 2

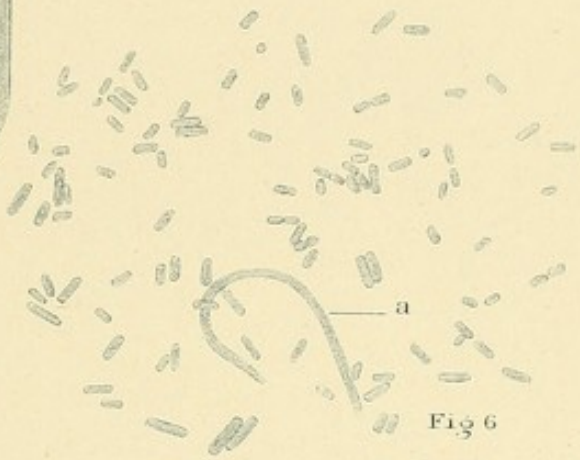

Fig 6

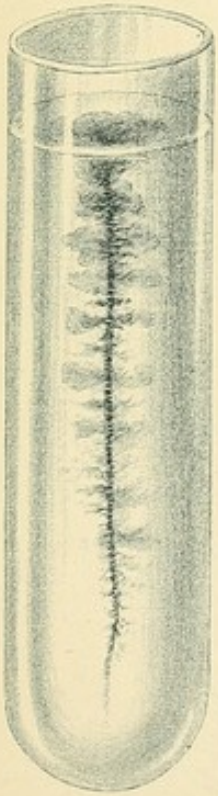

Fig 4

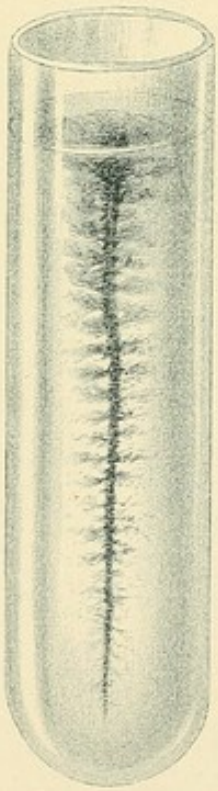

Fig 3

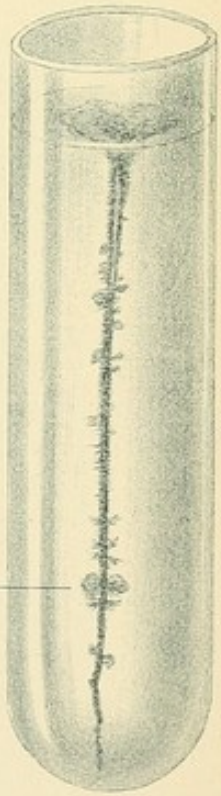

Fig 5

A. Hoen & Co. Lithocaustic, Baltimore.

PLATE VI.

FIG. 1. Bacillus *x* (Havana, 1889.) Smear preparation from surface of liver of rabbit 274, which died at end of 24 hours after receiving in cavity of abdomen 3 cubic centimeters of a bouillon culture of bacillus *x*. The bacilli are smaller than in recent cultures, but a pure culture was obtained from the peritoneal cavity of this rabbit in which the bacilli had the usual size as seen in Fig. 3. Fuchsin stain; × 1000.

FIG. 2. Bacillus *x*, from a potato culture. The potato had an acid reaction and the bacilli are unusually large. The culture was proved to be pure by making gelatine roll-tubes in which the colonies had the usual characters and the bacilli were of the usual dimensions. Fuchsin stain; × 1000.

FIG. 3. Bacillus *x*, from single colony in gelatine roll-tube. Fuchsin stain; × 1000.

FIG. 4. Bacillus *x*, from potato culture of 4 days. Fuchsin stain; × 1000.

FIG. 5. Bacillus *x*, colonies in gelatine roll-tube; three days at 20° C.; × 6 diameters.

FIG. 6. Bacillus *x*; colonies in gelatine roll tube; 48 hours at 22° C.; × 10 diameters.

FIG. 7. Bacillus *x*, culture in flesh-peptone-gelatine; 48 hours at 22° C.

PLATE VI.

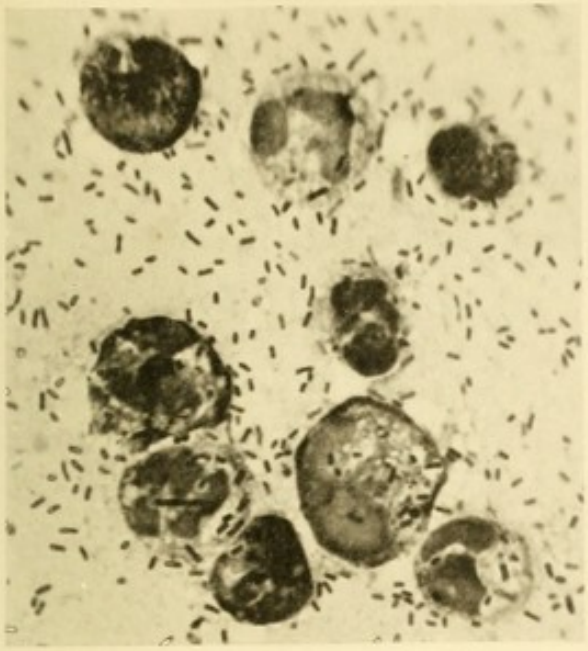

FIG. 1.

FIG. 2.

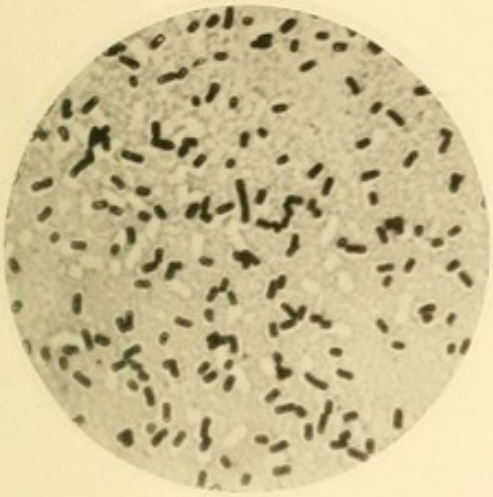

FIG. 3.

FIG. 4.

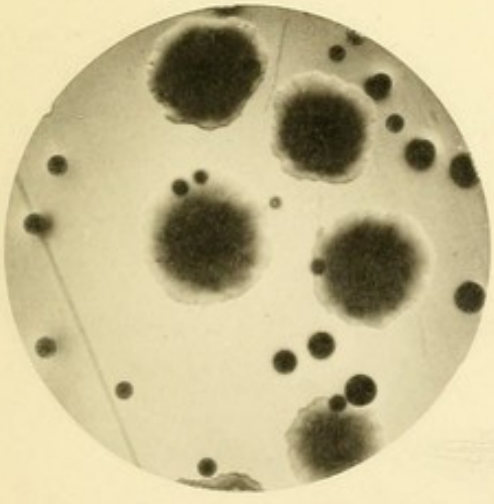

FIG. 5.

FIG. 7.

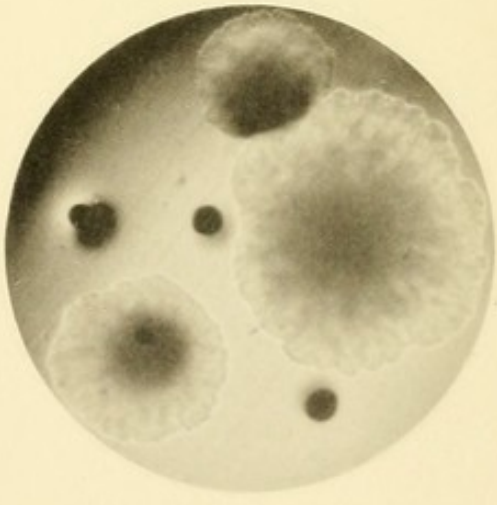

FIG. 6.

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PLATE VII.

FIG. 1. *Bacillus acidiformans* (Sternberg). From a potato culture; 48 hours at 22° C. Fuchsin stain; × 1000.

FIG. 2. *Bacillus acidiformans* in a leucocyte (phagocyte) from liver of rabbit inoculated with this bacillus. Fuchsin stain; × 1000.

FIG. 3. Group of leucocytes containing *B. acidiformans*, from blood of guinea-pig inoculated with a culture of this bacillus. Fuchsin stain; × 1000.

FIG. 4. Bacillus *a*, Havana, 1888. (*Bacterium coli commune.*) From a bouillon culture. Bismark brown stain; × 825.

FIG. 5. Colonies of *Bacterium coli commune* in gelatine roll tube; end of 48 hours, at 22° C. × 10 diameters.

FIG. 6. Single colony of *Bacillus* acidiformans, in gelatine roll tube, end of 2 weeks. × 10 diameters.

FIG. 7. *Bacillus acidiformans*, "stick" culture in glycerine-agar, showing the splitting up of the agar medium by the formation gas.

FIG. 8. *Bacillus acidiformans.* Culture in flesh-peptone-gelatine; end of 4 days, at 22° C.

PLATE VII.

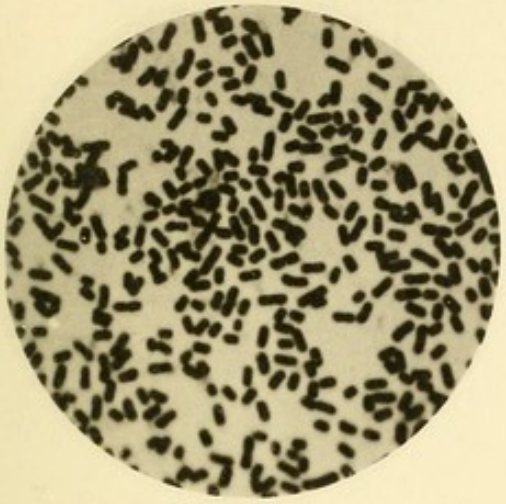


FIG. 1.

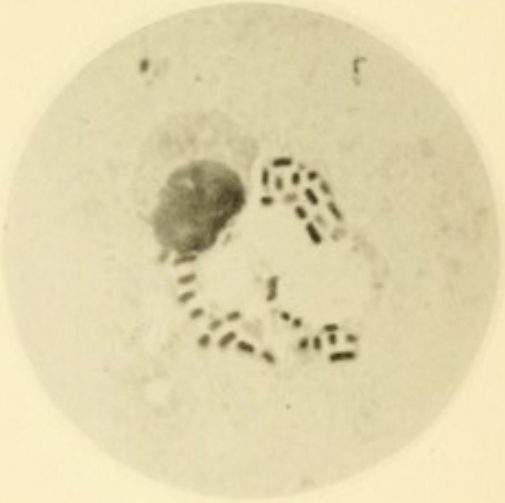


FIG. 2.

FIG. 7.

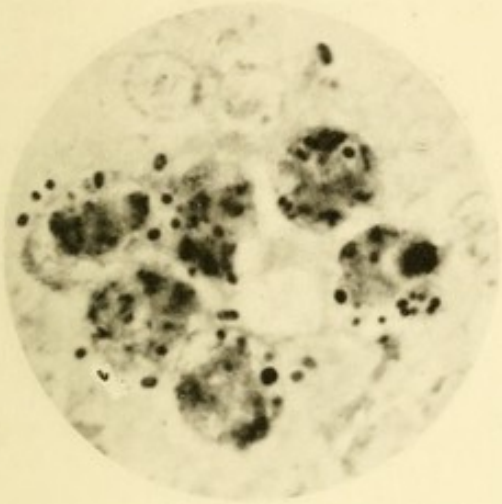


FIG. 3.

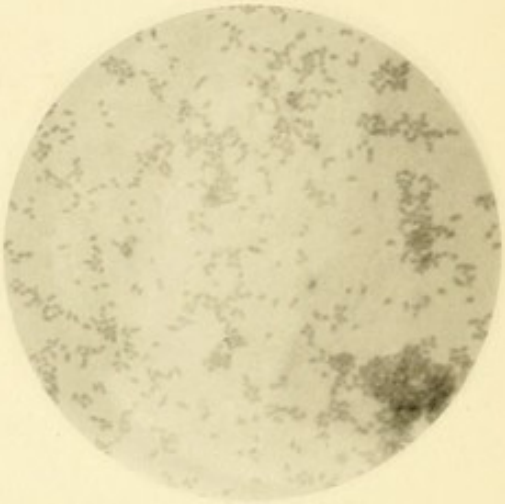


FIG. 4.

FIG. 8.

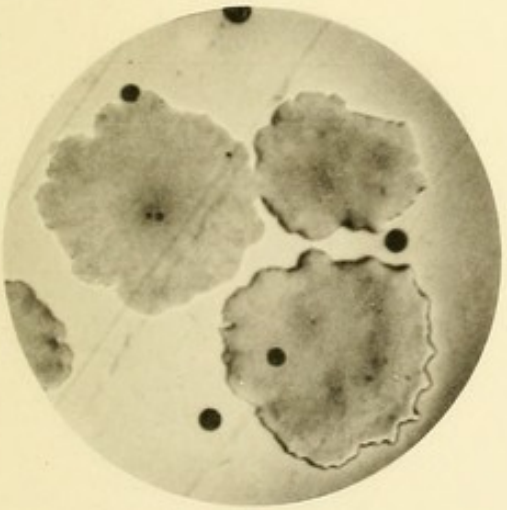


FIG. 5.

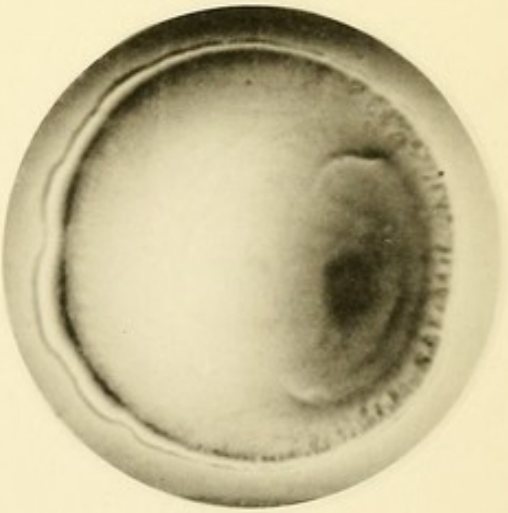


FIG. 6.

PLATE VIII.

FIG. 1. *Bacillus hepaticus fortuitus* (Sternberg). From an agar culture of 24 hours Fuchsin stain; × 1000.

FIG. 2. *Bacillus intestinus motilis* (Sternberg). From a potato culture, 10 days old. Fuchsin stain; × 1000.

FIG. 3. *Bacillus cavia fortuitus* (Sternberg). From potato culture of 9 days. Fuchsin stain; × 1000.

FIG. 4. *Bacillus caricida Havaniensis* (Sternberg). From a potato culture of 2 days. Fuchsin stain; × 1000.

FIG. 5. Colonies of *Bacillus hepaticus fortuitus* in flesh-peptone-gelatine roll-tube; end of 48 hours at 22° C. × 10.

FIG. 6. Colonies of *Bacillus intestinus motilis* in gelatine roll-tube; end of 24 hours, at 22° C. × 10.

FIG. 7. Culture in flesh-peptone-gelatine of *B. intestinus motilis*; end of 4 days, at 22° C.

FIG. 8. Culture in flesh-peptone-gelatine of *B. hepaticus fortuitus*; end of 4 days, at 22° C.

PLATE VIII.

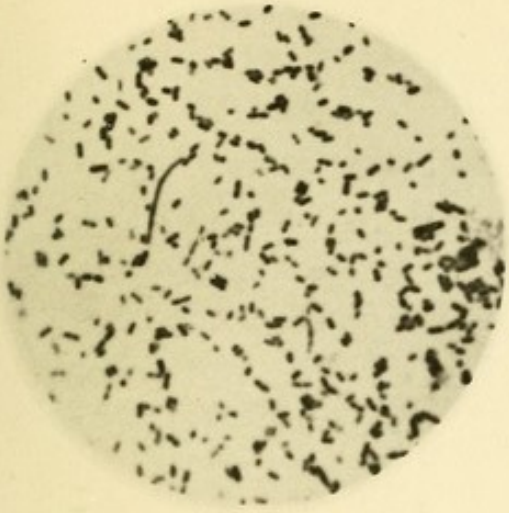
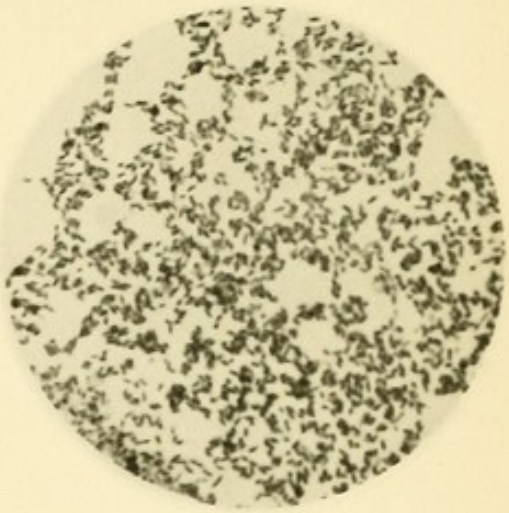


FIG. 1.



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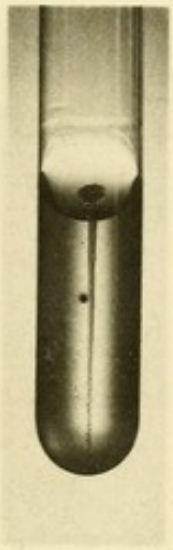
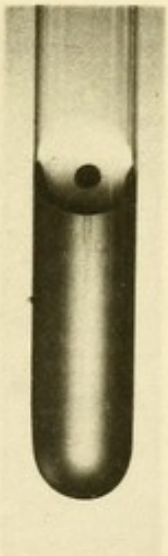


FIG. 7.

FIG. 3.

FIG. 4.



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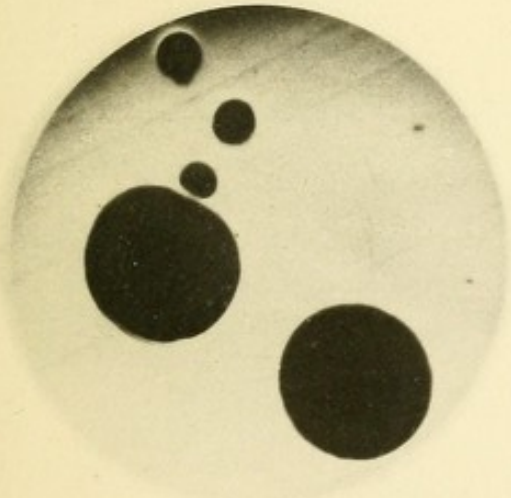

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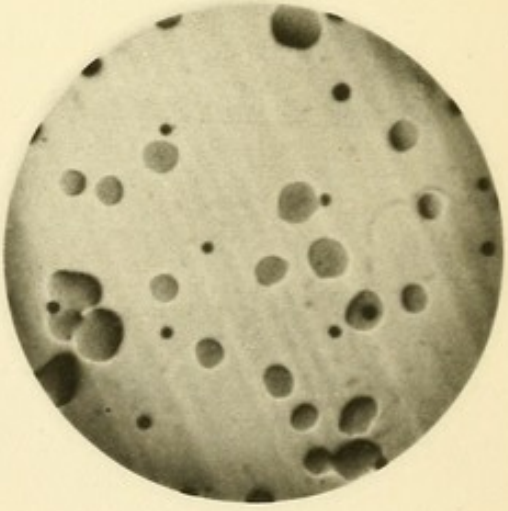


FIG. 6.

PLATE IX.

FIG. 1. *Bacillus Micrococcus Havaniensis* (Sternberg). Surface of agar culture, Fuchsin stain; × 1,000.

FIG. 2. *Bacillus fluorescens liquefaciens.* From single colony in gelatine roll-tube. Fuchsin stain; × 1,000.

FIG. 3. *Bacillus vacuolosis* (Sternberg). From potato culture, 4 days old. Fuchsin stain; × 1,000.

FIG. 4. *Bacillus vacuolosis,* from surface of agar culture, 6 days old; showing involution forms. Fuchsin stain; × 1,000.

FIG. 5. *Bacillus vacuolosis,* single colony in gelatine roll-tube; end of 5 days, at 20°C.; × 10.

FIG. 6. *Bacillus vacuolosis* culture in flesh-peptone-gelatine; end of 3 days, at 20°C.

PLATE IX.

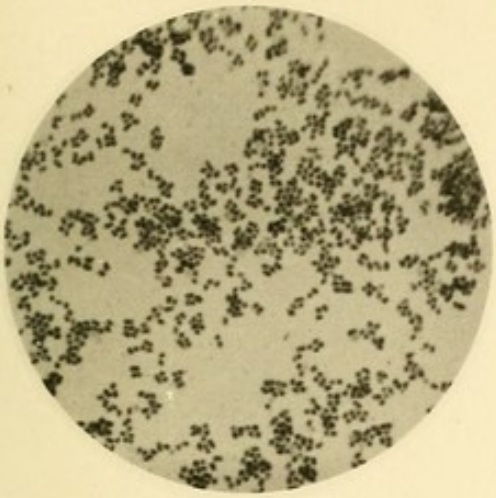


FIG. 1.

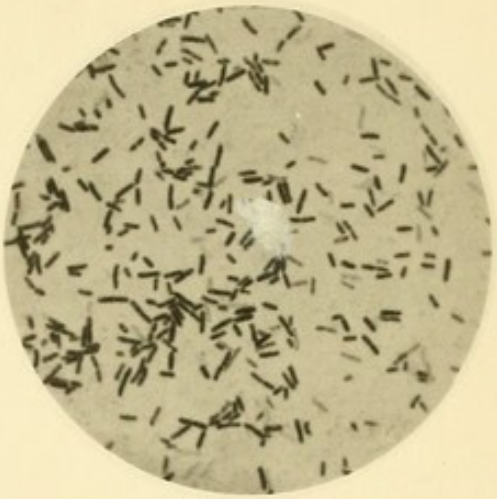
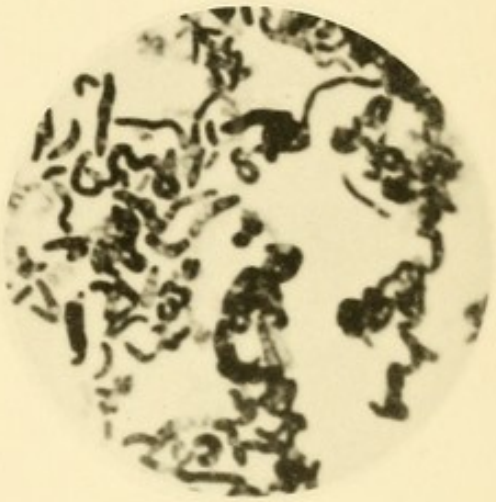


FIG. 2.

FIG. 3.



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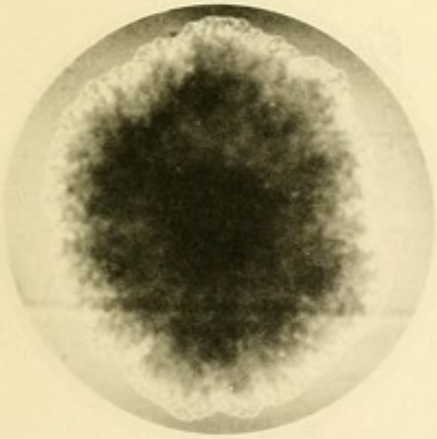

FIG. 5.

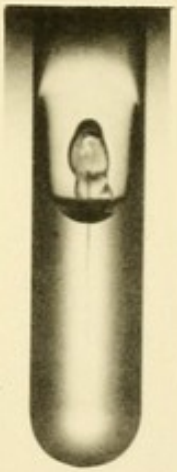

FIG. 6.

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PLATE X.

FIG. 1. *Bacillus pyocyamus* from potato culture of 24 nours. Fuschsin stain ; × 1,000.

FIG. 2. *Bacillus liquefaciens commune* (Sternberg). From gelatine culture. Fuchsin stain ; × 825 diameters.

Fig. 3. Colonies of *B. pyocyanus* in gelatine roll-tube; end of 2 days, at 20°C.; × 15.

FIG. 4. Liquefying colonies of B. pyocyanus in gelatine roll-tube; end of 3 days, at 20°C.; × 15.

FIG. 5. *B. pyocyanus* Culture in flesh-peptone-gelatine; end of 24 hours, at 22°C.

FIG. 6. *B. liquefaciens commune;* culture in flesh-peptone-gelatine; end of 24 hours, at 22°C.

PLATE X.

FIG. 1.

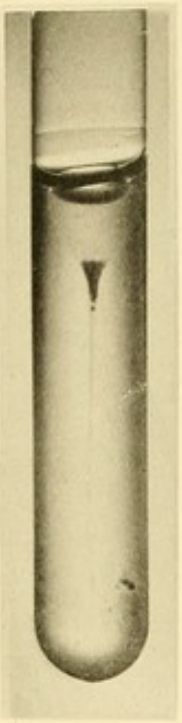


FIG. 5.

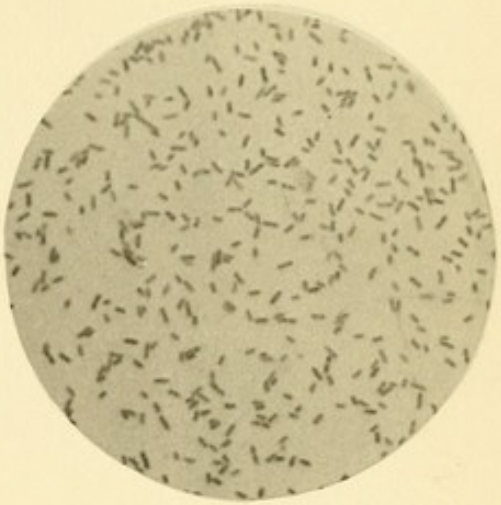


FIG. 2.

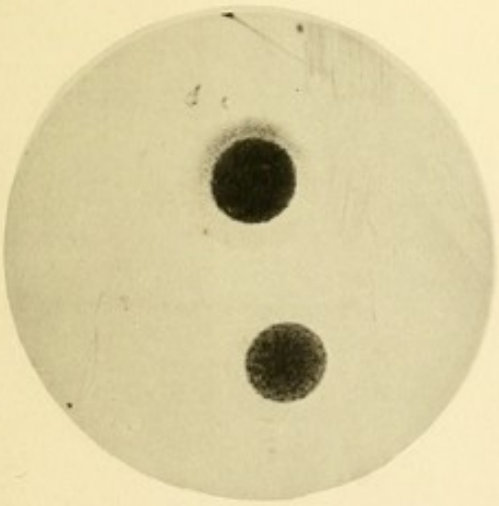


FIG. 3.

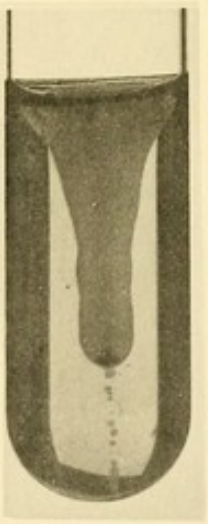


FIG. 6.

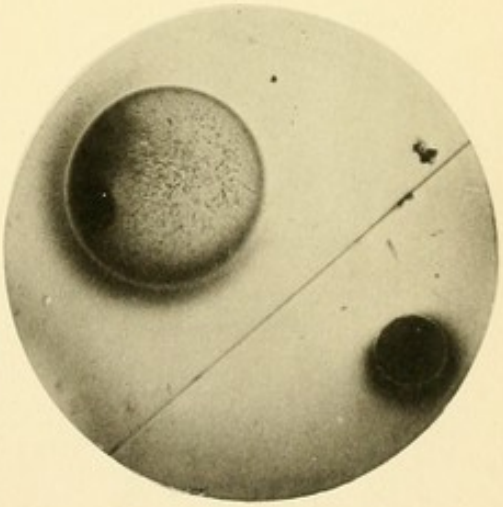


FIG. 4.

HELIOTYPE PRINTING CO. BOSTON.

PLATE XI.

FIG. 1. *Bacillus subtilis similis* (Sternberg). From potato culture of 5 days. Fuchsin stain; × 1000.

FIG. 2. Colonies of B. *subtilis similis* in gelatine roll tube; showing liquefaction of the gelatine; end of 48 hours, at room temperature, × 6.

FIG. 3. *Bacillus renalis fortuitus* (Sternberg). From gelatine culture. Fuchsin stain; × 1000.

FIG. 4. *Bacillus intestinus liquefaciens* (Sternberg). From a potato culture. Fuchsin stain; × 1000.

FIG. 5. *Bacillus intestinus liquefaciens.* Culture in flesh-peptone-gelatine; end of 24 hours, at 22° C.

PLATE XI.

FIG. 1.

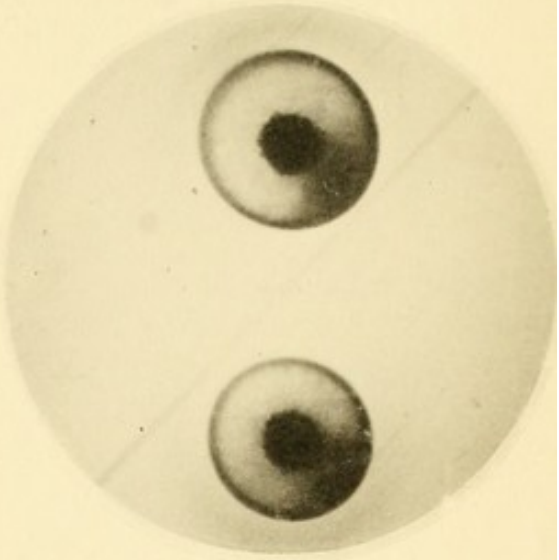


FIG. 2.

FIG. 5.

FIG. 3.

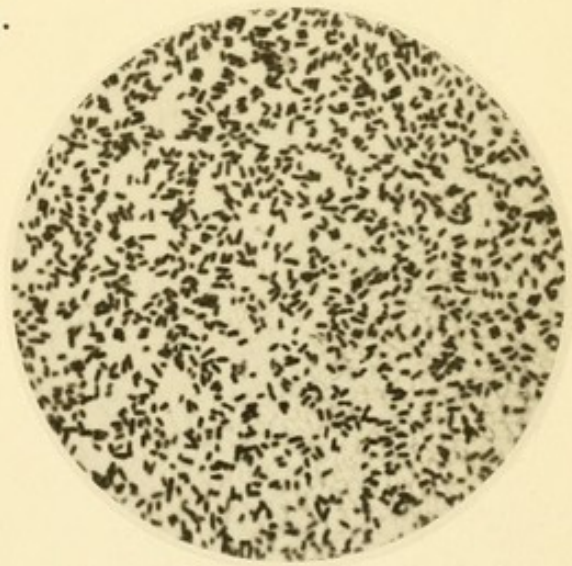


FIG. 4.

HELIOTYPE PRINTING CO. BOSTON.

PLATE XII.

FIG. 1. *Bacillus cadaveris* (Sternberg). Smear preparation from liver of yellow fever case, kept 48 hours in antiseptic wrapping. Fuchsin stain; × 1000.

FIG. 2. *Bacillus cadaveris* (Sternberg). From anaërobic culture in glycerine-agar roll-tube; contains also a micrococcus (see Fig. 1, Pl. XVII). Fuchsin stain × 1000.

FIG. 3. *Bacillus cadaveris*. From anaërobic culture in glycerine-agar roll-tube; × 1000.

FIG. 4. *Clostridium cadaveris* (Sternberg). From surface of an agar culture; × 1000.

PLATE XII.

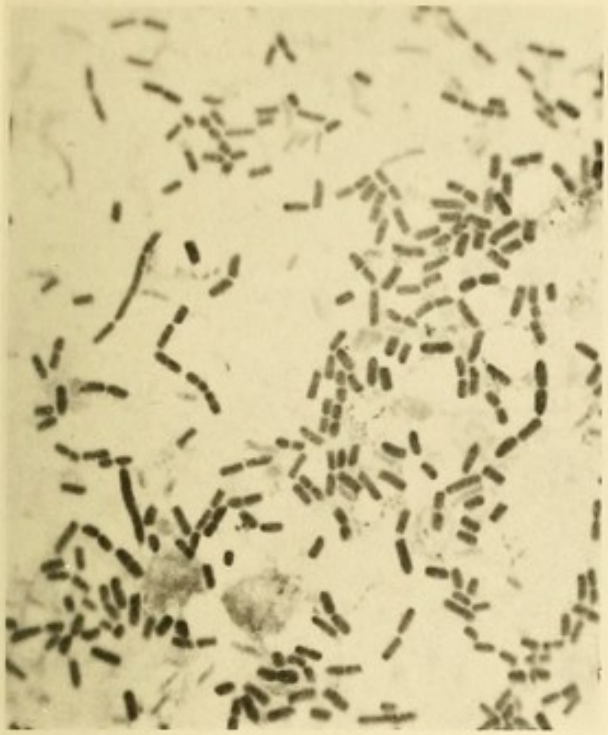
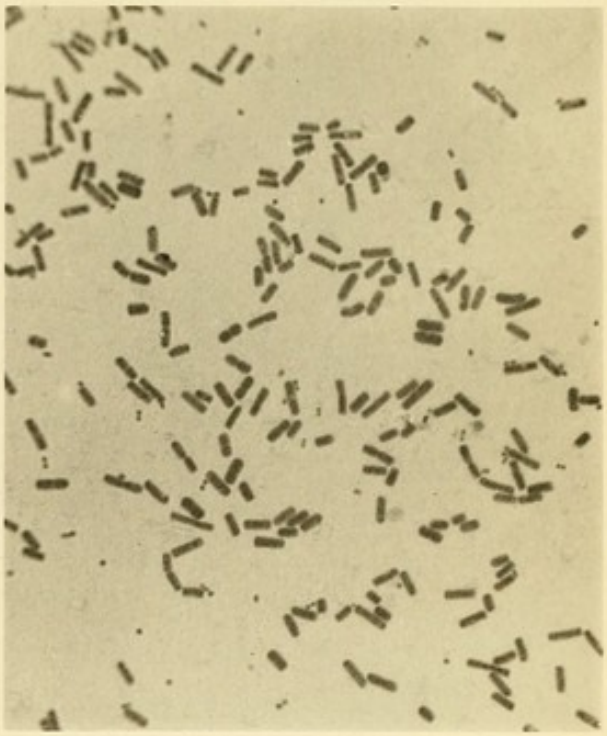


FIG. 1.



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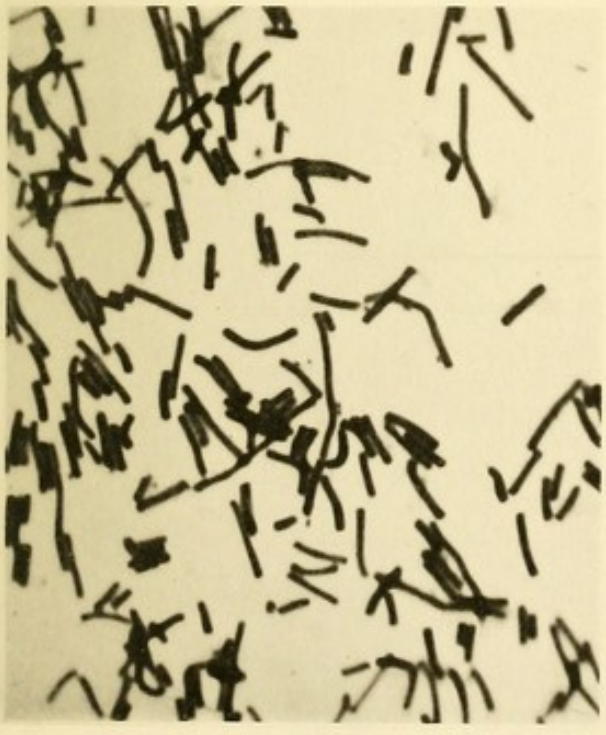


FIG. 3.

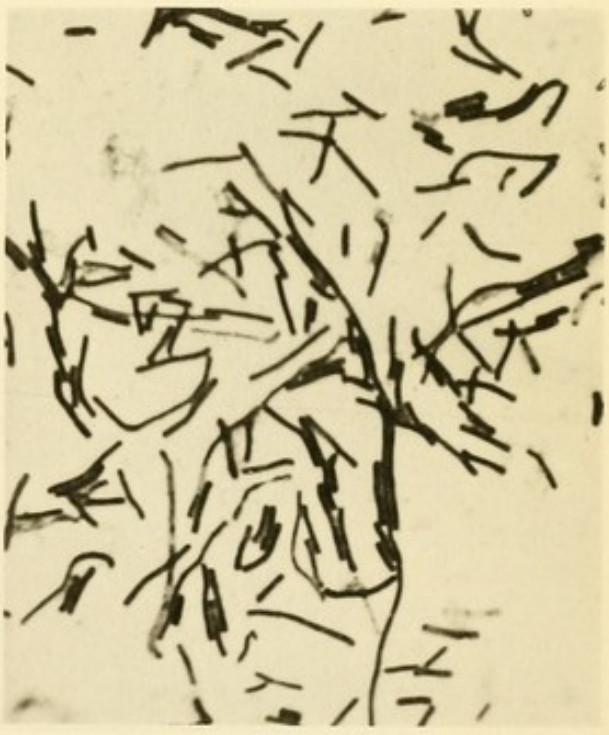


FIG. 4.

PLATE XIII.

FIG. 1. *Bacillus filiformis* (Sternberg). From surface of agar culture; fuchsin stain; × 1,000.

FIG. 2. *Bacillus Martinez* (Sternberg). From single colony in glycerine-agar roll-tube; fuchsin stain; × 1,000.

FIG. 3. Colonies of B. filiformis in glycerine-agar roll-tube; end of 5 days at 35° C.; × 10.

FIG. 4. Colonies of B. Martinez in gelatine roll-tube at end of 4 days; × 10.

FIG. 5. Culture of B. Martinez in flesh-peptone gelatine; end of 4 days at 22° C.

FIG. 6. Culture of B. filiformis in flesh-peptone gelatine; end of 7 days at 22° C.

PLATE XIII.

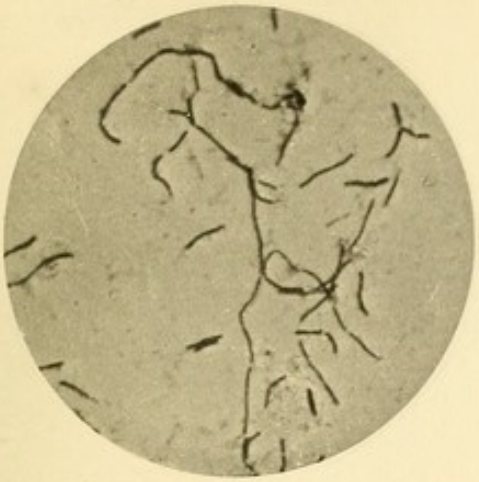


FIG. 1.

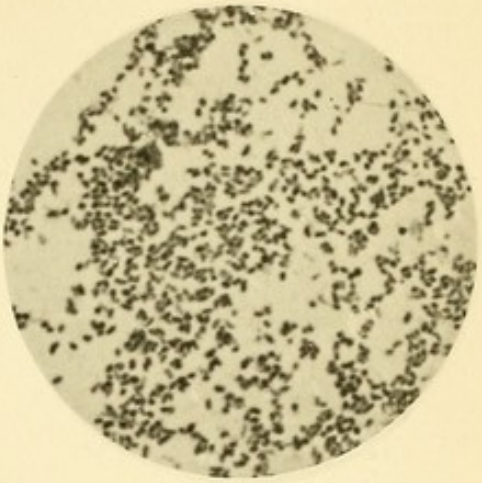


FIG. 2.

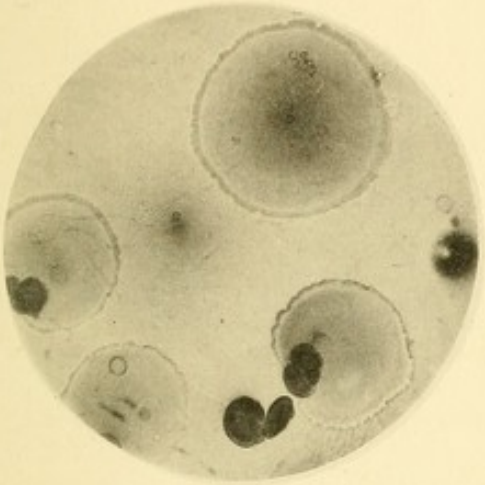


FIG. 3.

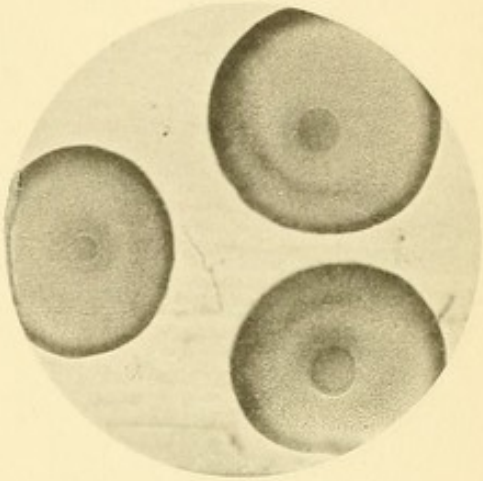


FIG. 4.

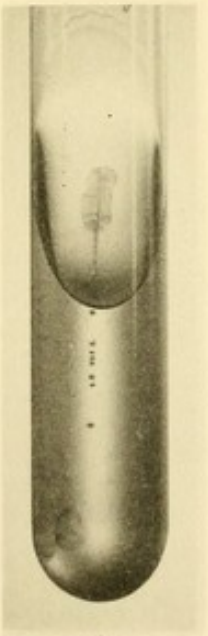


FIG. 5.

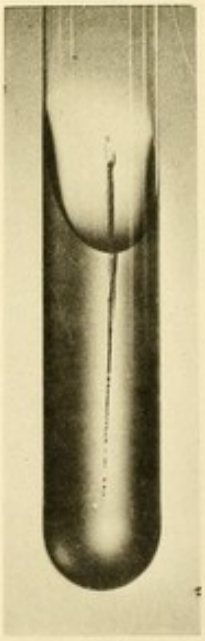


FIG. 6.

HELIOTYPE PRINTING CO. BOSTON.

PLATE XIV.

FIG. 1. *Bacillus I*, Havana, 1889. From gelatine culture; fuchsin stain; × 1,000.
FIG. 2. *Bacillus E*, Havana, 1889. From gelatine culture; fuchsin stain; × 1,000.
FIG. 3. *Bacillus A*, Havana, 1889. From potato culture; fuchsin stain; × 1,000.
FIG. 4. *Bacillus renalis fortuitus* (Sternberg.) From gelatine culture; fuchsin stain; × 1,000.
FIG. 5. *Bacillus U*, Havana, 1889. From single gelatine colony; fuchsin stain; × 1,000.
FIG. 6. *Bacillus Y*, Havana, 1889. From single gelatine colony; fuchsin stain; × 1,000.
FIG. 7. Single colony *Bacillus I.;* end of 24 hours at 20°C. × 10.
FIG. 8. *Bacillus I*, culture in flesh-peptone gelatine; 48 hours at room temperature.

PLATE XIV.

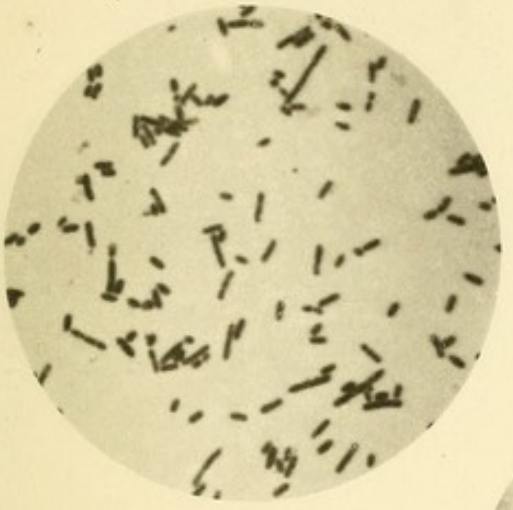


FIG. 1.

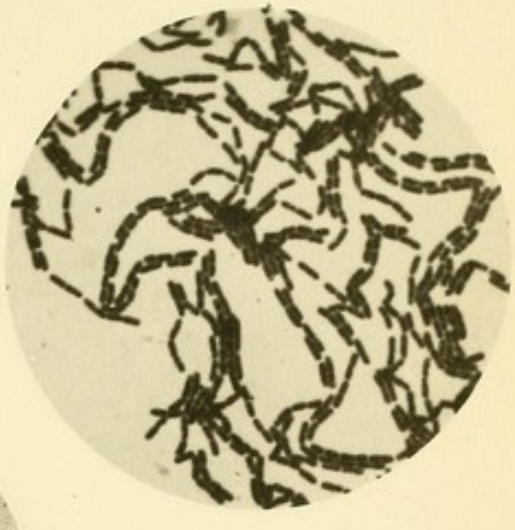


FIG. 2.

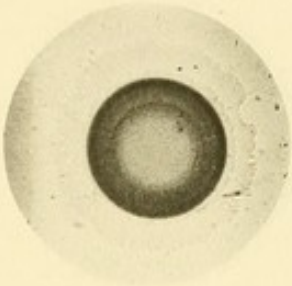


FIG. 7.

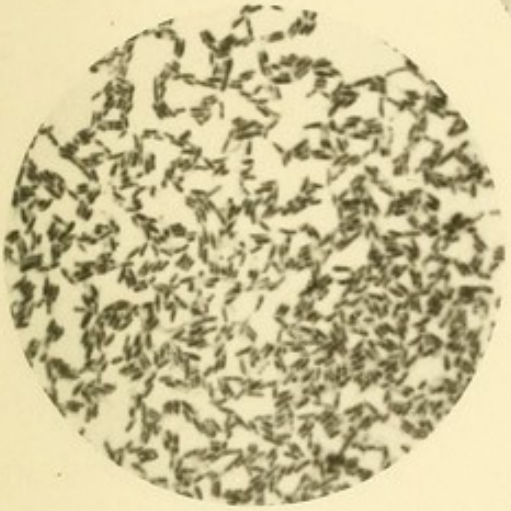
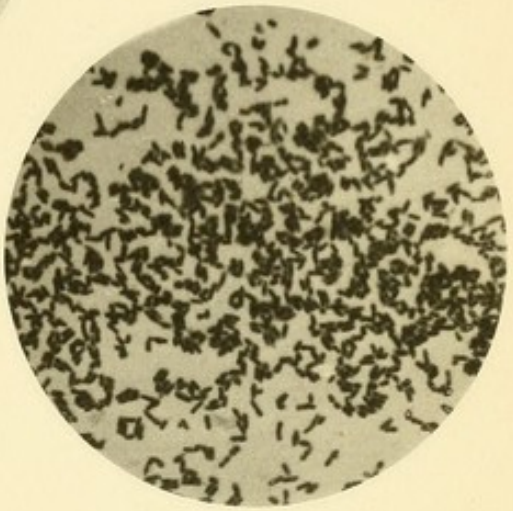


FIG. 3.



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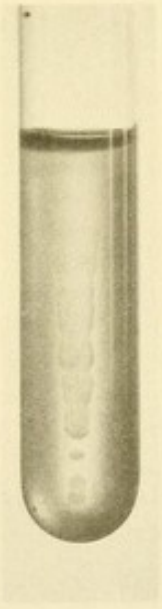


FIG. 8.

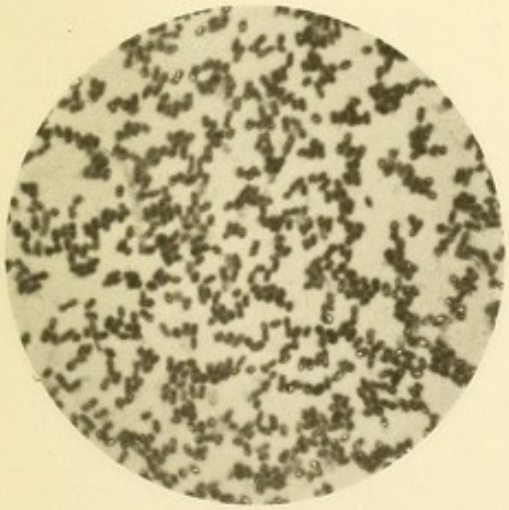


FIG. 5.

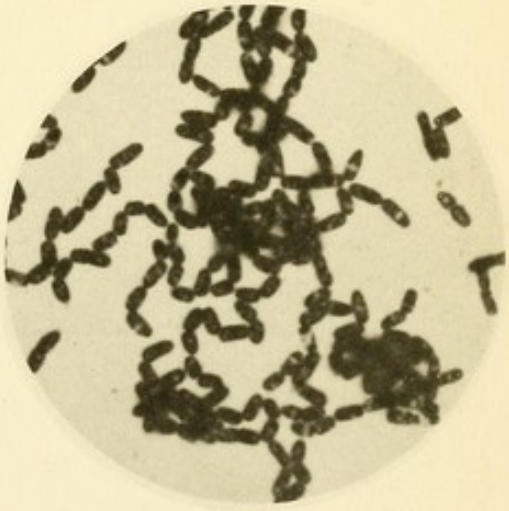


FIG. 6.

HELIOTYPE PRINTING CO., BOSTON.

PLATE XV.

FIG. 1. *Bacillus B*, Havana, 1889. From bottom of long agar "stick culture;" fuch sin stain; × 1,000.

FIG. 2. *Bacillus K*, Havana, 1889. From gelatine culture; × 1,000.

FIG. 3. *Bacillus C*, Havana, 1889. From gelatine culture; × 1,000.

FIG. 4. *Bacillus L*, Havana, 1889. From potato culture, 4 days old; × 1,000.

FIG. 5. *Bacillus G*, Havana, 1889. From single gelatine colony; × 1,000.

FIG. 6. Slender bacillus from specimen of Freire's vaccine, brought from Rio de Janeiro by Dr. Lane in a sealed glass tube. The mount was made immediately after opening the tube; stained with gentian violet; × 1,000.

PLATE XV.

FIG. 1.

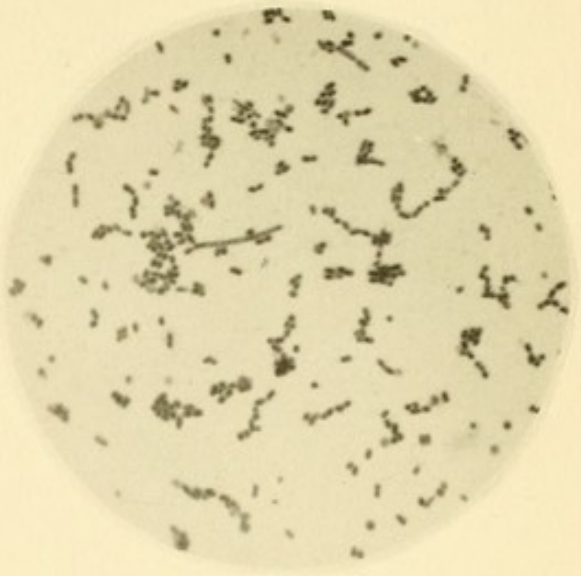


FIG. 2.

FIG. 3.

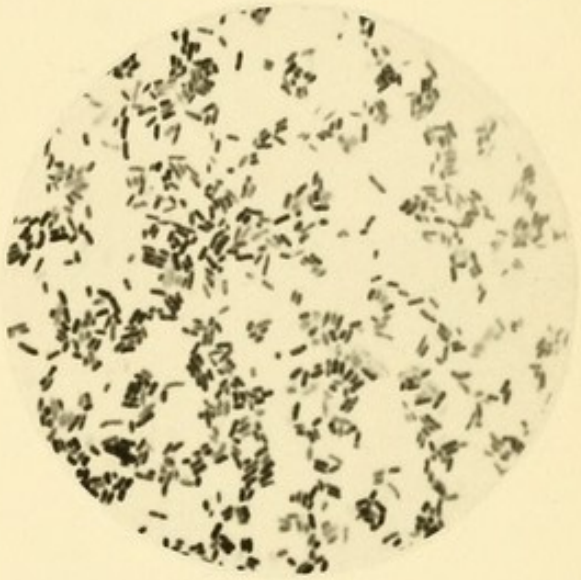


FIG. 4.

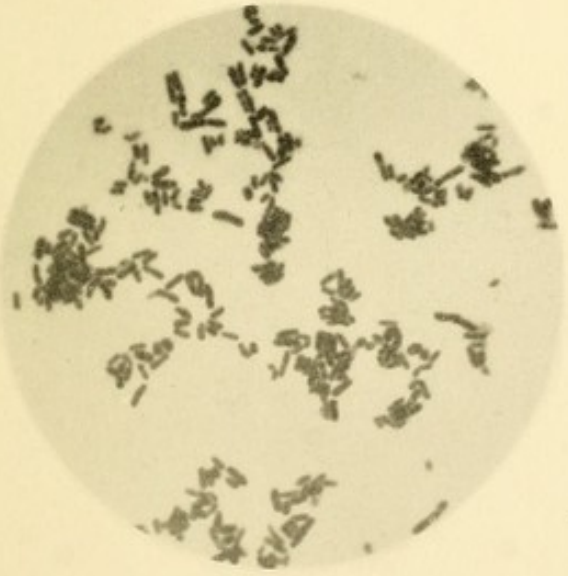


FIG. 5.

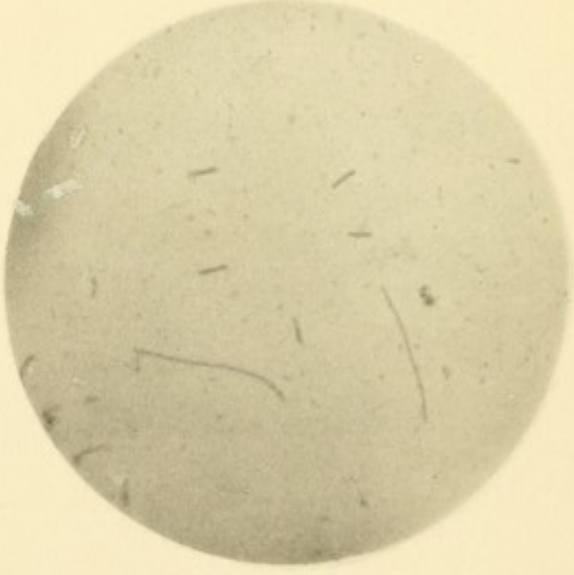


FIG. 6.

HELIOTYPE PRINTING CO. BOSTON.

PLATE XVI.

FIG. 1. *Bacillus gracilis* (Sternberg). From single colony in gelatine roll-tube; × 1,000.

FIG. 2. *Bacillus coli similis* (Sternberg). From single colony in gelatine roll-tube; × 1,000.

FIG. 3. *Bacillus gracilis.* Colonies in gelatine roll-tube at end of 24 hours at 22° C.; × 10.

FIG. 4. *Bacillus coli similis.* Colonies in gelatine roll-tube at end of 24 hours at 22° C.; × 12.

FIG. 5. *Bacillus gracilis.* Colonies in gelatine roll-tubes at end of 48 hours at 22° C.; × 12.

FIG. 6. *Bacillus coli similis.* Culture in flesh-peptone-gelatine at end of 7 days at 20 C.

FIG. 7. *Bacillus coli similis.* Colonies in gelatine roll-tube at end of five days at 20° C.; × 12.

PLATE XVI.

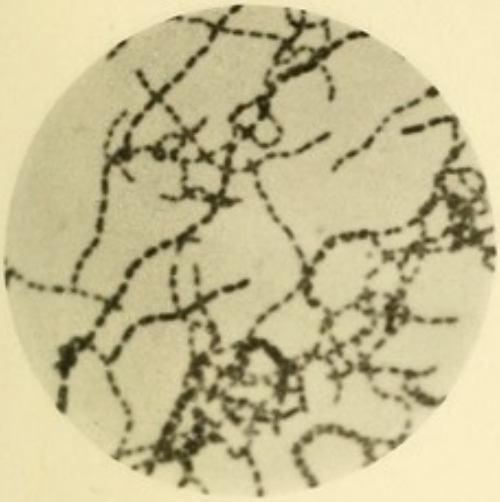


FIG. 1.

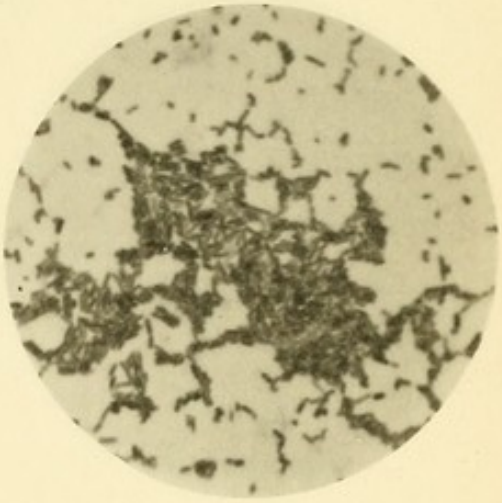


FIG. 2.

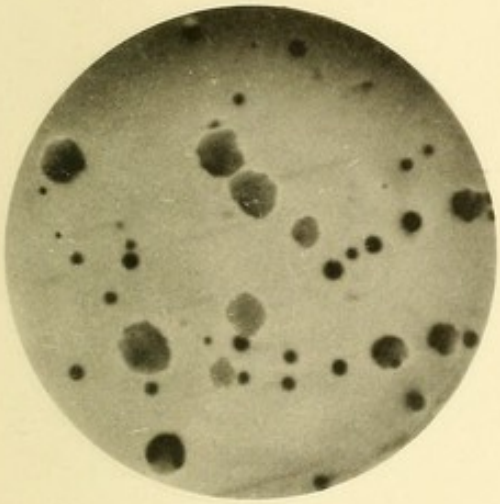


FIG. 3.

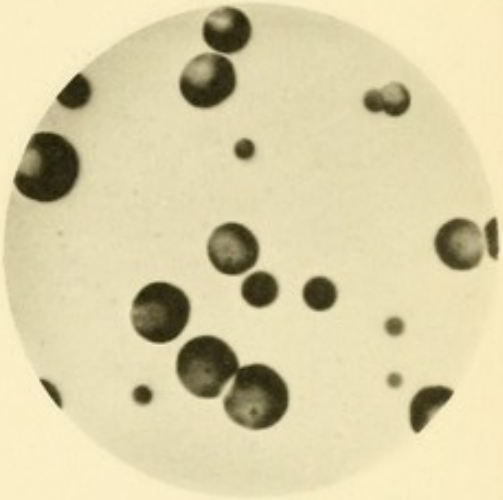
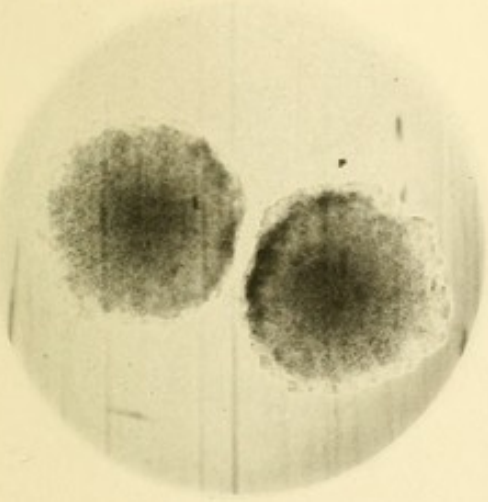


FIG. 4.



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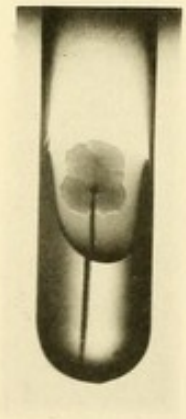


FIG. 6.

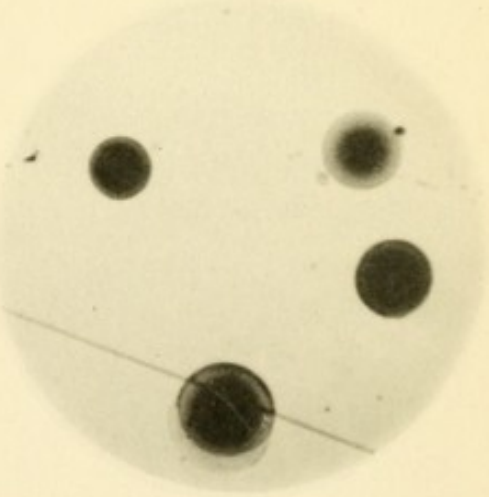


FIG. 7.

HELIOTYPE PRINTING CO. BOSTON.

PLATE XVII.

FIG. 1. *Streptococcus cadaveris* (Sternberg) (Streptococcus pyogenes?). From culture in agua coco; × 1,000.

FIG. 2. *Streptococcus Havaniensis.* From vomit (not black) kept in collecting tube for 24 hours. Yellow-fever case in military hospital, Havana, 1889; × 1,000.

FIG. 3. *Micrococcus luteus.* From gelatine culture; × 1,000.

FIG. 4. *Streptococcus liquefaciens* (Sternberg). From anaërobic culture in flesh-peptone gelatine; × 1,000.

FIG. 5. Colony in gelatine roll-tube of *Streptococcus liquefaciens;* × 10.

FIG. 6. *Streptococcus cadaveris.* Colonies in gelatine roll-tube; ×10.

FIG. 7. *Streptococcus liquefaciens.* Culture in flesh-peptone-gelatine; end of 7 days at 22° C.

PLATE XVII.

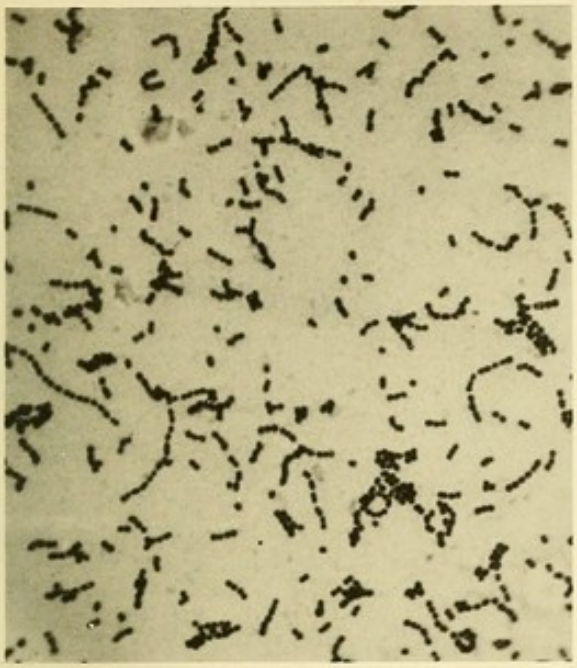


FIG. 1.

FIG. 2.

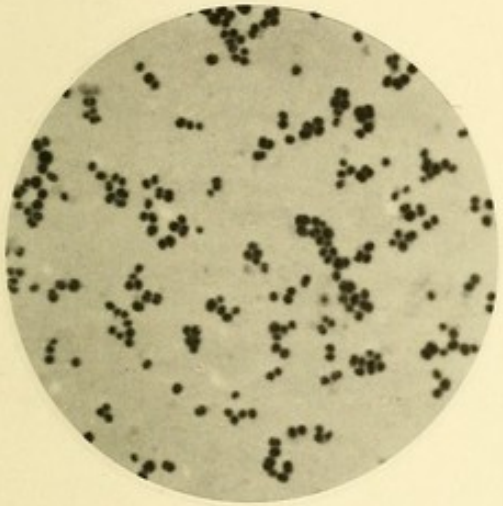


FIG. 3.

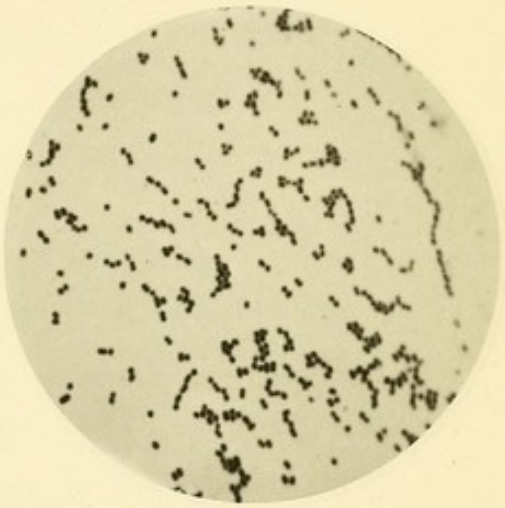


FIG. 4.

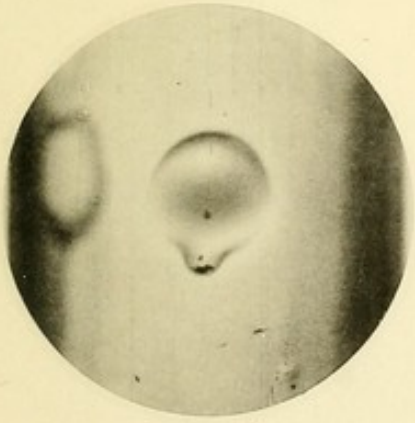


FIG. 5.

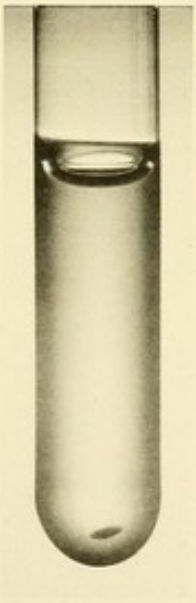


FIG. 7.

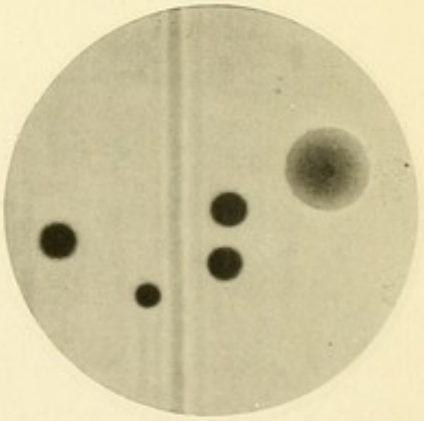


FIG. 6.

HELIOTYPE PRINTING CO., BOSTON

PLATE XVIII.

FIG. 1. *Torula gastricus* (Sternberg). From surface of agar culture. Not stained; × about 400.

FIG. 2. *Micrococcus hepaticus* (Sternberg). From surface of agar culture. Bismarck brown stain; × 825.

FIG. 3. *Micrococcus J.* (Havana, 1888); × 1,000. Obtained from liver of case 8, kept 48 hours in an antiseptic wrapping.

FIG. 4. *Micrococcus Finlayensis* (Sternberg). From surface of agar culture. Bismarck brown stain; × 825.

FIG. 5. *Micrococcus versatilis albus* (Sternberg). From a culture in agua coco. Fuchsin stain; × 1,000.

PLATE XVIII.

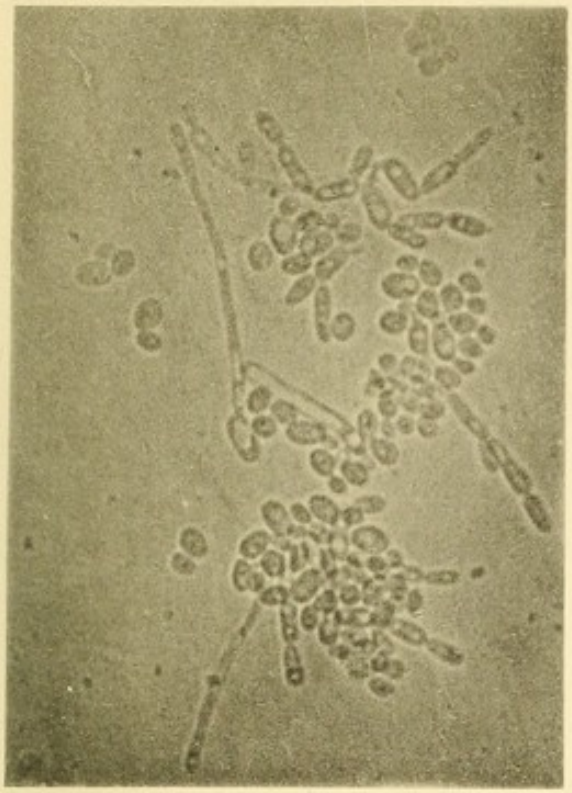


FIG. 1.

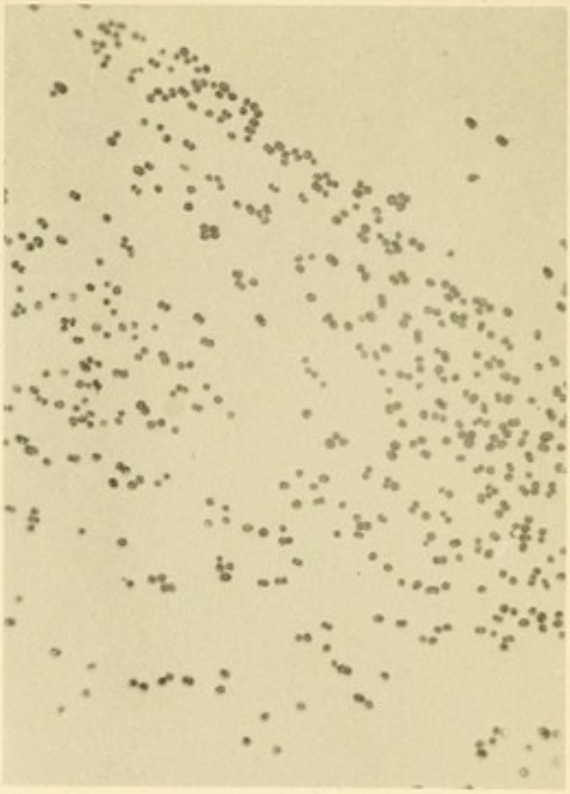


FIG. 2.

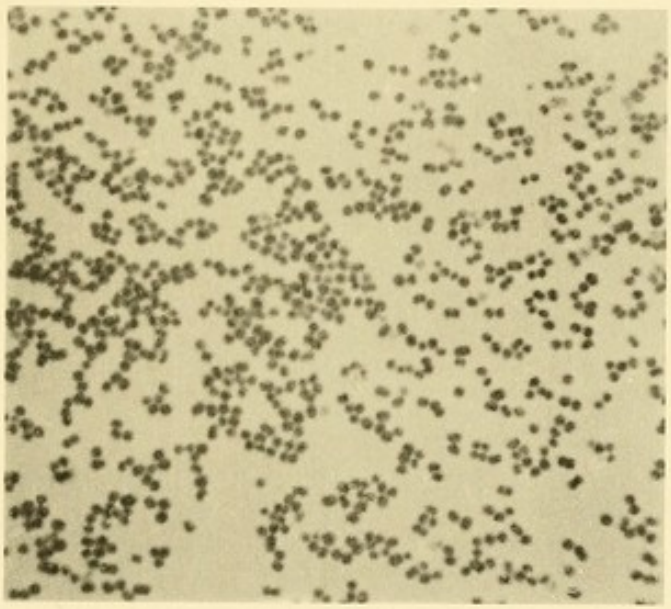
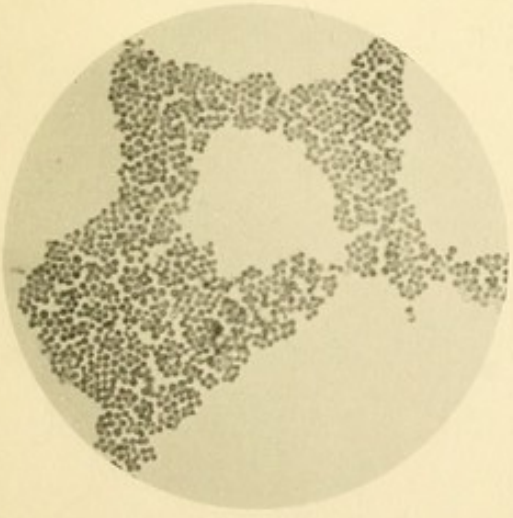


FIG. 3.



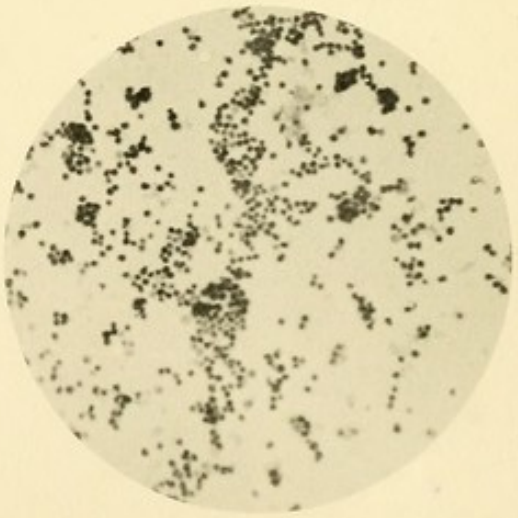


PLATE XIX.

FIG. 1. *Bacillus Havaniensis* (Sternberg). Potato culture at end of 15 days at room temperature.

FIG. 2. *Bacillus pyocyanus.* Agar culture at end of 5 days at room temperature.

FIG. 3. *Bacillus pyocyanus.* Potato culture at end of 5 days.

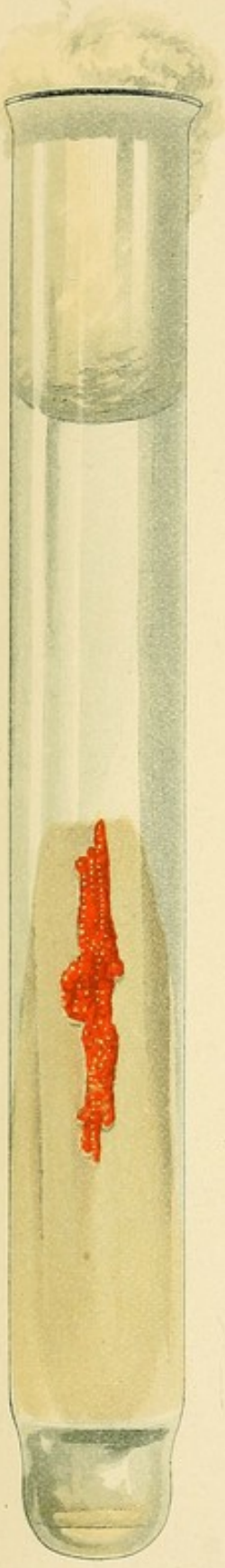


Fig 1

Fig 2

Fig 3

A. Hoen & Co. Lithocaustic, Baltimore.

PLATE XX.

FIG. 1. *Micrococcus tetragenus versatilis* (Sternberg). Agar culture at end of 10 days, at 22° C.

FIG. 2. *Micrococcus of Freire.* Agar culture at end of 7 days, at 22° C.

FIG. 3. *Bacillus of Gibier.* Agar culture at end of 12 days, at 22° C.

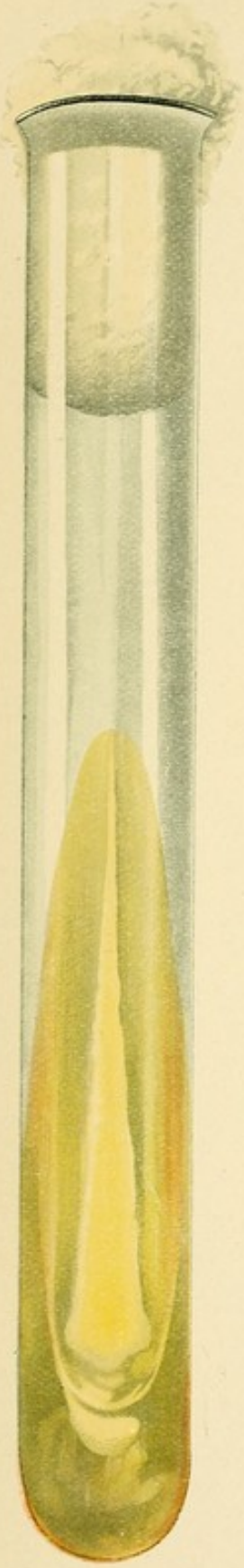


Fig 1

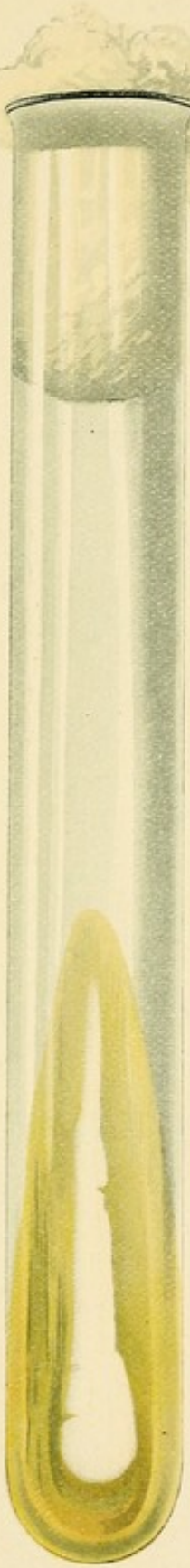


Fig 2

Fig 3

A. Hoen & Co. Lithocaustic, Baltimore.

PLATE XXI.

FIG. 1. *Bacillus fluorescens liquefaciens.* Potato culture at end of 1 month at room temperature.

FIG. 2. *Bacillus fluorescens liquefaciens.* Gelatine culture at end of 1 month at room temperature.

FIG. 3. *Bacillus liquefaciens commune* (Sternberg.) Potato culture at end of 20 days at room temperature.

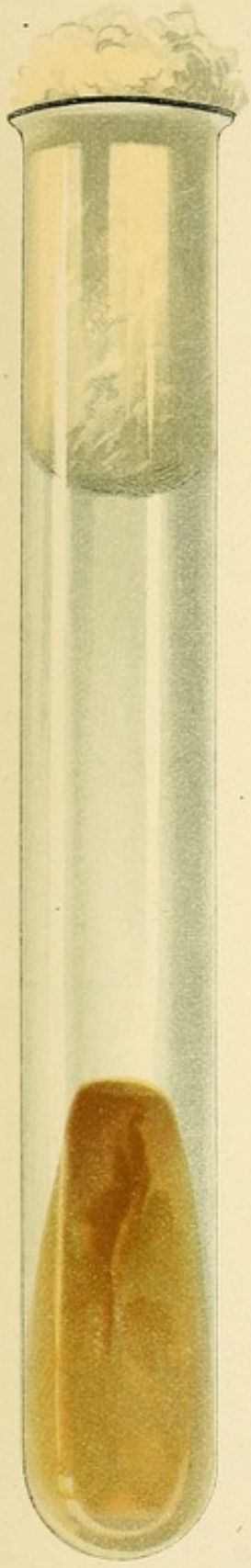


Fig 1

Fig 2

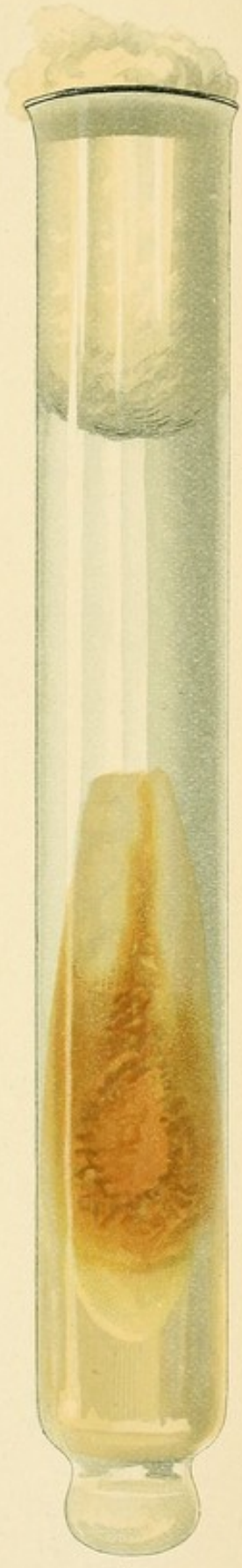


Fig 3

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Annex