TREASURY DEPARTMENT

U. S. MARINE HOSPITAL SERVICE

REPORT

OF

COMMISSION OF MEDICAL OFFICERS

DETAILED BY AUTHORITY OF THE PRESIDENT

TO INVESTIGATE

THE CAUSE OF YELLOW FEVER

1899

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WASHINGTON:
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INTRODUCTORY.

LETTERS ADDRESSED TO THE SECRETARY OF THE TREASURY, RECOMMENDING AN INVESTIGATION BY OFFICERS OF THE MARINE-HOSPITAL SERVICE INTO THE CAUSE OF YELLOW FEVER, WITH SPECIAL REFERENCE TO THE REPORTED DISCOVERY OF THE YELLOW FEVER GERM BY PROFESSOR SANARELLI.

TREASURY DEPARTMENT,
OFFICE SUPERVISING SURGEON-GENERAL
MARINE-HOSPITAL SERVICE,
Washington, D. C., August 30, 1897.

The SECRETARY OF THE TREASURY.

SIR: I have the honor to invite your attention to the announcement of Dr. Guiseppe Sanarelli, of Montevideo, who alleges to have discovered the specific cause of yellow fever. The researches leading to this discovery were published first in full in the annals of the Pasteur Institute.

On carefully analyzing his results, especially his comparative experiments, one is bound to come to the conclusion that they are the researches bearing the stamp of a painstaking and conscientious observer.

It appears more than probable that Dr. Sanarelli has discovered the germ of yellow fever. The claims made by Sanarelli are of such a character that they are difficult to refute, even if there were a desire to do so, by reason of the fact that they are marked with thoroughness and clearness which admits but little question, and although some of the statements made with regard to the etiology and pathology and prophylaxis of yellow fever are in striking contrast with the present preconceived notions, they are not sufficient to invalidate his claims.

Should his alleged discovery prove to be true, it is one of the greatest boons which has been given to the world since Koch announced the cause of tuberculosis and of cholera, and may now make it possible to apply even more successful measures in the prevention and treatment of yellow fever than of other acutely contagious diseases.

The discovery is one of the most vital interest and importance to the United States. More than 20,000,000 of our population live in a section of country which possesses all the natural conditions for the disease to become epidemic if once introduced. To prevent this the Government is compelled to maintain large and numerous quarantine establishments for the purpose of guarding against its introduction, and entailing expenses and hardship upon our maritime commerce.

By this discovery it seems more than probable that many of the restrictions now imposed may be dispensed with, since it is possible to deal with a known entity.

With these possibilities in view it would seem advisable to send an expert to investigate and confirm the claims of Dr. Sanarelli in order that the advantages of this alleged discovery may be made available to the people.

I have therefore respectfully to recommend that an officer of the Marine-Hospital Service, P. A. Surg. H. D. Geddings, be detailed for this duty.

Dr. Geddings is now in Europe where, at the Pasteur Institute, he has been engaged in a special course of bacteriological research, and has already made a preliminary study of the germ discovered by Sanarelli. He is an expert bacteriologist, has had a large experience both in the observation and treatment of cases of yellow fever, has himself had the disease, and is eminently fitted for this duty.

It is suggested that Passed Assistant Surgeon Geddings be sent to Montevideo to study the germ under the direction of Sanarelli, and afterwards, should it be thought necessary, to visit the places where yellow fever is epidemic, viz, Santos and Rio Janeiro, where he can make confirmatory studies upon the cases, arrive at independent conclusions concerning it, and acquire a practical knowledge of the application of this great discovery.

The expense connected with this investigation would be small, being confined to the officer's actual expenses, and with the approval of the President could be paid from the fund for the prevention of epidemic diseases.

I have the honor to remain, very respectfully, yours,

WALTER WYMAN,
Supervising Surgeon-General, M. H. S.

Approved.
L. J. GAGE, *Secretary.*

TREASURY DEPARTMENT,
OFFICE SUPERVISING SURGEON-GENERAL,
MARINE-HOSPITAL SERVICE,
Washington, D. C., November 8, 1897.

The SECRETARY OF THE TREASURY.

SIR: I respectfully invite your attention to my letter of August 30, 1897, requesting authority to send Passed Assistant Surgeon Geddings to Montevideo to make investigations into the reported discovery of the germ of yellow fever by Dr. Sanarelli, and to subsequently pursue investigations into the cause of this disease at Santos, Rio de Janeiro, or other yellow-fever-infected ports. This letter was duly approved by yourself, and was prepared also for the approval of the President. The letter was not presented to the President immediately, as he was out of the city. In the meantime, within a few days, the present epidemic of yellow fever in the South was announced at Ocean Springs, Miss., and the detail of Dr. Geddings was held in abeyance, because his services were immediately required in the yellow-fever district. Later, while on duty in New Orleans, he was directed to pursue the investigations, taking advantage of the yellow-fever epidemic in that city. Passed Assistant Surgeon Wasdin, who made the first diagnosis of the yellow fever in the present epidemic, and who himself contracted the disease and recovered therefrom, was also detailed for like service. Dr. Geddings, in a report dated November 1, states that Dr. Wasdin and himself have carried their investigations to a point where it is desirable that they should be continued in some more favorable locality. Authority is therefore requested to detail these two officers for duty in the port of Havana, where yellow fever exists during the whole year, and where

exceptional advantages may be had for the scientific investigation into the cause of the disease.

These two officers are especially qualified for this investigation.

P. A. Surg. Eugene Wasdin has made a special study of bacteriology, and was professor of pathology for four years in Charleston, S. C., in the Medical College of South Carolina.

As previously stated, he made the first diagnosis of yellow fever at Ocean Springs, and insisted thereon in the face of much opposition, and verified it by post-mortem examination.

Passed Assistant Surgeon Geddings is an expert in bacteriology and biological chemistry, and is attached to the hygienic laboratory of the Service. Several years ago he was ordered to Johns Hopkins Hospital, and the Bureau was given a written assurance by the director of the laboratory of his competency to conduct original investigations.

In later years he has been frequently called upon for special work of this character, his latest detail being as technical delegate on the part of the United States to the International Sanitary Conference held at Venice during the months of February and March, 1897.

After the adjournment of the Venice conference Dr. Geddings was engaged in special laboratory work in the Pasteur Institute in Paris and in Koch's laboratory in Berlin, paying special attention to the discoveries of Sanarelli, as stated in my letter of August 30.

The discovery of the cause of yellow fever is a matter of vital interest and importance to the United States, as it would result in obviating large expense, and place the preventive measures upon a strictly scientific basis, and prevent needless panic and such paralyzation of commerce and traffic as has prevailed during the past two months.

Furthermore, it may be possible not only to ameliorate the conditions affecting commerce, but in the near future to apply preventive and curative measures to the individual.

I, therefore, respectfully request approval of this detail, the expenses for which can legally and appropriately be paid from the epidemic fund.

Respectfully, yours,

WALTER WYMAN,
Supervising Surgeon-General M. H. S.

Approved.
L. J. GAGE, *Secretary.*

Approved.
WILLIAM MCKINLEY.

LETTER TRANSMITTING REPORT.

TREASURY DEPARTMENT,
OFFICE SUPERVISING SURGEON-GENERAL,
MARINE-HOSPITAL SERVICE,
Washington, D. C., July 26, 1899.

The SECRETARY OF THE TREASURY.

SIR: Referring to my letters of August 30 and November 8, 1897, I have to transmit herewith the report of the commission of medical officers of the Marine-Hospital Service detailed by your direction and that of the President to investigate the nature of yellow fever.

This work was begun by Surg. (then P. A. Surg.) Eugene Wasdin, in accordance with Bureau order of September 11, 1897. Dr. Wasdin, who had made the first diagnosis of yellow fever at Ocean Springs, Miss., and had himself contracted and recovered from the disease, was directed to make investigation into the cause of yellow fever as indicated by Sanarelli, and was furnished by the Bureau with a bacteriological outfit for this purpose.

On October 4 he was relieved from duty at Ocean Springs and ordered to New Orleans, where the opportunities for investigation were greater, and on the 6th of October P. A. Surg. H. D. Geddings was ordered from Jackson, Miss., to New Orleans for the same purpose. These two officers were directed to work together or independently, as might be agreed between them, and they conducted all the work conjointly. Notice of this investigation was published in Public Health Reports of October 8, 1897.

November 1 they reported they had carried their investigations to a point where it was desirable to continue them in some more favorable locality, and accordingly their formal detail for duty in the port of Havana was requested and approved by yourself and the President, mention of this detail being made in Public Health Reports of November 12, 1897.

December 1, 1897, the two officers arrived in Havana, obtained suitable rooms, established a laboratory, and continued their investigations until March, 1898, when, on account of threatened hostilities with Spain, they were returned to the United States and their services engaged in quarantine work during the ensuing summer and fall. In the meantime they submitted a preliminary report upon the work already accomplished, which was published in the Public Health Reports of November 11, 1898.

November 8, 1898, both officers were directed to return to Havana and resume the investigation, which had been suspended on account of the war with Spain. They continued their labors in the laboratory, which had remained undisturbed during the war, until June of the present year, when they informed the Bureau they had arrived at definite conclusions, and requested that they be ordered to Washington to prepare a report, which follows.

The findings of this commission, verifying the discovery made by Sanarelli, and making still further advances than did Sanarelli himself by determining the specificity of the bacillus icteroides, and that the primary infection of yellow fever is received through the respiratory tract, in other words, verifying one discovery and making others of almost equal importance, at the same time eliminating incorrect theories, must be considered a notable achievement in medical science and one of great practical value to the people of the United States and other countries infected or liable to be infected by yellow fever.

The findings of the commission, also, with regard to the susceptibility of the bacillus icteroides to germicidal influences, and the possibility of the production of an antitoxic serum which may operate as does the antitoxin in diphtheria, both to prevent and cure the disease, are matters of the greatest interest and importance. The preparation of the antitoxic serum by improved methods and with a hope of obtaining a serum of sufficient potency to be of practical value has already been begun in the hygienic laboratory of the service.

In view of the widespread interest which this report will excite and the practical deductions to be drawn therefrom, I have respectfully to request authority to have the same printed.

Respectfully, yours,

WALTER WYMAN,
Supervising Surgeon-General M. H. S.

Approved.
L. J. GAGE,
Secretary of the Treasury.

REPORT.

WASHINGTON, D. C., *July 10, 1899.*

SIR: In compliance with your order of the 11th of November, 1897, instructing us to proceed as a commission, appointed "by order of the President," for the purpose of investigating the cause of yellow fever to Havana, Cuba, and there to make all necessary arrangements for the prosecution of this investigation, we have the honor to report that your instructions have been fully and faithfully carried out, the investigation having been pursued until definite results were obtained, and we now ask to be allowed to submit these results, giving in detail an account of the technical work upon which our observations were based, and from which the following conclusions have been drawn:

CONCLUSIONS.

First. That the microorganism discovered by Prof. Guiseppe Sanarelli, of the University of Bologna, Italy, and by him named "bacillus icteroides," is the cause of yellow fever.

Second. That yellow fever is naturally infectious to certain animals, the degree varying with the species; that in some of the rodents local infection is very quickly followed by blood infection, and that, while in dogs and rabbits there is no evidence of this subsequent invasion of the blood, monkeys react to the infection the same as man.

Third. That infection takes place by way of the respiratory tract, the primary colonization in this tract giving rise to the earlier manifestations of the disease.

Fourth. That in many cases of the disease, probably a majority, the primary infection or colonization in the lungs is followed by a "secondary infection," or a secondary colonization of this organism in the blood of the patient. This secondary infection may be complicated by the coinstantaneous passage of other organisms into the blood, or this complication may arise during the last hours of life.

Fifth. There is no evidence to support the theory advanced by Professor Sanarelli that this disease is primarily a septicæmia, inasmuch as cases do occur in which the bacillus icteroides can not be found in the blood, or organs in which it might be deposited therefrom.

Sixth. That there exists no causal relationship between the bacillus "X" of Sternberg and this highly infectious disease; and that this bacillus, "X," is frequently found in the intestinal content of normal animals and of man, as well as in the urine and the bronchial secretion.

Seventh. That, so far as your commission is aware, the bacillus icteroides has never been found in any body other than of one infected with yellow fever; and that whatever may be the cultural similarities between this and other microorganisms, it is characterized by a specificity which is distinctive.

Eighth. That the bacillus icteroides is very susceptible to the influences injurious to bacterial life, and that its ready control by the processes of disinfection, chemical and mechanical, is assured.

Ninth. That the bacillus icteroides produces *in vitro* as well as *in vita* a toxin of the most marked potency; and that, from our present knowledge, there exists a reasonable possibility of the ultimate production of an antiserum more potent than that of Professor Sanarelli.

ACKNOWLEDGMENTS.

We are indebted to the State and War Departments of our Government for the permission obtained to pursue our investigations in the Spanish province of Cuba and to continue the same after occupation by the United States forces. We especially acknowledge the courtesy of Governor-General Blanco and Secretary-General Congosto of the Spanish province of Cuba, and of the gentlemen of the Spanish medical staff, from whom we at all times received valuable assistance.

To Major-General Brooke, U. S. A., commanding the military division of Cuba, and to his chief of medical staff, Col. R. M. O'Reilly, and to Surgeon-Majors Gorgas and Davis, of the Department of Havana, we owe acknowledgment for numerous courtesies and aid in the prosecution of our work. We are also indebted to Maj. Gen. Fitzhugh Lee, U. S. V., for invaluable assistance while serving as our consul-general to Cuba.

We also acknowledge gratefully the spirit of friendly interest always evinced toward us by the medical profession of the city of Havana, and the assistance proffered and given us. Especially do we remember the courtesies received from the Academy of Sciences of Havana and from the staff of the laboratory of the "Cronica Medica Quirurgica," and its distinguished chief, Dr. Santos Fernandez.

Our acknowledgments are also due to our esteemed friend, the distinguished scientist, Dr. Carlos Finlay, who placed us under many pleasant obligations, and to the medical staffs of the various city hospitals of Havana.

Finally, we would express our appreciation of the interest of the President and of the Secretary of the Treasury, as indicated by their ready assent to our detail, and of your own active sympathy and support.

We have the honor to remain, respectfully yours,

EUGENE WASDIN,
Surgeon, M. H. S.

H. D. GEDDINGS,
Passed Assistant Surgeon, M. H. S.

SURGEON-GENERAL MARINE-HOSPITAL SERVICE,
Washington, D. C.

THE CAUSE OF YELLOW FEVER.

Since the discovery of the pathogenicity of the vegetable microorganisms, or bacteria, numberless propositions have been advanced in favor of this property in organisms found associated frequently with the acute infectious diseases, and since many of these claims were based upon insufficient data and merely served to obscure investigation, and to retard, there resulted from the hands of that master in bacteriology, Robert Koch, in 1881, a series of requirements or postulates, each of which must of necessity be fulfilled by one claiming specificity for a new organism in a given disease and which, but slightly altered, are generally acknowledged to-day. In order that an organism may maintain the claim of specificity Koch requires:

1. The organism must be present in the blood or tissues of men or animals sick with or recently dead with the disease.
2. The organism must be isolated, freed from all foreign bodies or substances, and be procured in pure culture.
3. This pure culture introduced into susceptible animals must produce the disease in question.
4. In the disease thus produced the organism must be found distributed as in the natural disease.

And to these may be added a fifth requirement, viz:

5. That the chemical products of the organism when introduced into the tissues must produce symptoms and effects (but not necessarily the lesions) of the disease.

Have all of these requirements been fulfilled in the case of every acute, specific, infectious, or contagious disease? The answer must be, in all fairness, no. A certain class of diseases, certainly specific, clearly communicable, have not as yet yielded to research any specific microorganism, and by these diseases are meant the so-called eruptive fevers, as smallpox, chicken pox, measles, scarlet fever, and rötheln.

The claim of Sanarelli, being the most recent as well as the most scientific claim as to the cause of yellow fever, naturally has occupied our attention to the exclusion of all others, especially in view of the fact that in 1890[1] Sternberg effectually disproved all such prior claims. During the epidemic of yellow fever along the gulf littoral of Mississippi, and in New Orleans, La., in 1897, especial effort was put forth by ourselves[2] and other observers[3] to isolate, if possible, the Sanarelli organism.

The results of that work have already been placed before you.[2] Immediately upon our establishment in Havana it became our effort to control the work of Sanarelli by a repetition of his experiments as given in his "memoirs,"[4] as well as to continue the search for the organism in all cases of yellow fever open to our study, and to control

[1] Sternberg, report on yellow fever, 1890; Treasury Department, Document 1328, Marine Hospital Service.

[2] Preliminary report of commission, November, 1898, Appendix B, C, and D. See also Public Health Reports, vol. xiii, Nos. 45, 46, and 47.

[3] Archinard, New York Medical Journal, January, 1899.

[4] Annales de l'Institute Pasteur, 1897, June and November.

these cases by a series of observations in cases of and in bodies dead from other known diseases.

In the fulfillment of the first postulate of Koch, Sanarelli made use of 13 cases, isolating the organism in 7, or in 54 per cent. In New Orleans, during the epidemic of 1897, we obtained cultures from a number of cases and isolated the bacillus icteroides in 83.33 per cent of the cases, all of the work, save that of collection, being done in our laboratory in Havana, Cuba.

Owing to the impracticability of obtaining a record of the cases from which we isolated the bacillus icteroides in New Orleans, La., we must ask that you accept the diagnosis made in these cases by those competent to do so without a relation of the cases themselves. For your information we give in detail the histories of cases studied in Havana, regretting the impossibility of obtaining at times records of temperature and pulse rate, since such records were not deemed essential, even in the Spanish military hospitals, and the civil institutions were badly demoralized.

Case No. 1.—Seen in the military hospital at Regla, January, 1898. Name, X. Y.; aged about 35 years; a native of Spain; residence in Cuba two years, in the country districts.

History of an acute chill occurring at night, followed by high temperature; much pain in back and head and muscles of limbs, nausea, and vomiting.

On the fifth day of the disease the status presented was as follows: Facies—dull and heavy appearance of face and eyes, the former suffused, latter are yellow and injected; skin of body congested and decidedly yellow; gums congested and bleed on touch; much epigastric tenderness; some nausea, and, on the preceding day, some "black vomit;" temperature, 38.50;[1] pulse, 75; urine albuminous. Blood taken from ear tip in bulbs, the bulbs sown in bouillon, and this plated. From these plates the organism was readily isolated in pure culture. This case was seen but the once, and finally recovered. Temperature record and notes were promised us but were never given.

The organisms associated with b. icteroides in this case were b. proteus vulgaris and b. coli communis.

Case No. 2.—Seen at the military hospital at Regla, February 24, 1898. Name, Pablo S. Ligura; age, 23 years; a native of Spain; four years' residence in Cuba; declares he has had yellow fever two years before.

There is a history of malarial cachexia, much prostration and some emaciation, an obscure attack of chill and rise of temperature at night, with following chills.

Status presens: There is facial congestion; eyes are clear; tongue broad and lightly coated; herpes labialis; slight delirium; no trace of albumen in urine; no headache, but lumbago and pain in limbs; temperature, 37.4; pulse, 90; much epistaxis.

Our diagnosis was malarial fever. Blood from this case gave only a skin contamination.

Case No. 3.—Seen in company with Dr. Jacobson at the Quinta de Dependientes. History and appearance indicated a malarial attack. There was recurring chilliness; an irregular fever; albuminuria, and nausea and vomiting. There was also an opinion that it was the fever "de borras." We were decidedly of the opinion that it was not yellow fever.

Blood taken and successively plated and replated, but the only organism encountered was b. coli com., possibly of contamination from skin.

Case No. 4.—Juan Yljarbe Arguiñano, aged about 30 years; seen at the military hospital of San Ambrosio, February 13, 1898.

[1] The temperatures recorded in this report are in the centigrade scale.

History: Residence in Cuba two years; served in Spanish army in the rural districts; in Havana three months. Became sick on the 10th with chill, and intense backache, headache, and pains in the limbs; nausea was incessant.

Status on 13th: Face flushed a dark red; eyes dull and yellow; pupils dilated; tongue furred with red edges; gums bleeding; skin of chest deeply congested; pain over liver and epigastrium; pain in head and eyeballs and back; temperature, 38°; pulse, 72; urine highly albuminous; dark and offensive stools. Diagnosis of yellow fever concurred in.

Blood taken from the ear on the third day of the disease planted in bouillon and plated gave a number of colonies of bac. icteroides. This case ended in recovery.

Case No. 5.—Jose Canaldas Caldet, aged 28 years, a native of Spain, was seen at the military hospital of San Ambrosio February 13, 1898.

There is a history of two years' army service in Cuba; he became sick in hospital while convalescent from venereal disease; there was a sharp chill, headache and backache, nausea and vomiting on the 9th.

Status on the 13th: The face is dull and yellow; eyes are suffused and yellow; there are bloody sordes on the teeth, and dried blood about alae nasi; the odor is pronounced; skin of body yellow and congested; some petechiæ on trunk; urine contains 25 per cent of moist albumen; the stools are black; temperature, 37.8°; pulse, 60.

There was no bedside record, but the interné told our interpreter that this want of correlation between pulse and temperature had been noted for two days.

Blood taken from ear tip in bulbs on the fourth day of the disease; plates gave numerous colonies of bac. icteroides, associated with b. coli communis.

NOTE.—Of these 5 cases we agreed in the diagnosis of 3, No. 1 at Regla, and No. 4 and No. 5 at San Ambrosio, and in these 3 the Sanarelli organism was easily found. In the 2 others the most diligent search failed to find the organism in the blood.

Case No. 6.—The case of Doane; aged about 50; a native of the United States.

History: Arrived in Havana on December 27, 1898, and was in service at the custom-house; was taken sick January 5, in the evening, with chill and intense head and back pains; nausea and retching; temperature rose to 39.8° C. (Dr. Castellanos) on the 9th; pulse low; always much epigastric tenderness; diagnosis of yellow fever.

Status on the 12th: The face is flushed from stasis; the eyes injected; conjunctivæ a bright yellow; skin of scalp (gray hair) a canary yellow; gums swollen and easily bleed; the tongue is read at tip and edges; the sordes are bloody; the skin of trunk a dusky yellow; urine contains 75 per cent of moist albumen; there is almost an anuria; the stools are tarry and offensive. He died at 10 p. m. of the 12th.

We drew blood from the ear tip in Sternberg bulbs (seventh day of disease), and succeeded in isolating the bac. icteroides in pure culture.

Case No. 7.—Private Patrick Smith, Eighth Infantry, United States Army; aged about 28 years; was seen in regimental hospital at the "Punta," a notably bad locality in Havana.

There is a history of malarial intoxication at Santiago during the Shafter campaign, and he had been under treatment for it recently. The present attack of illness came on on the 5th of February, 1899.

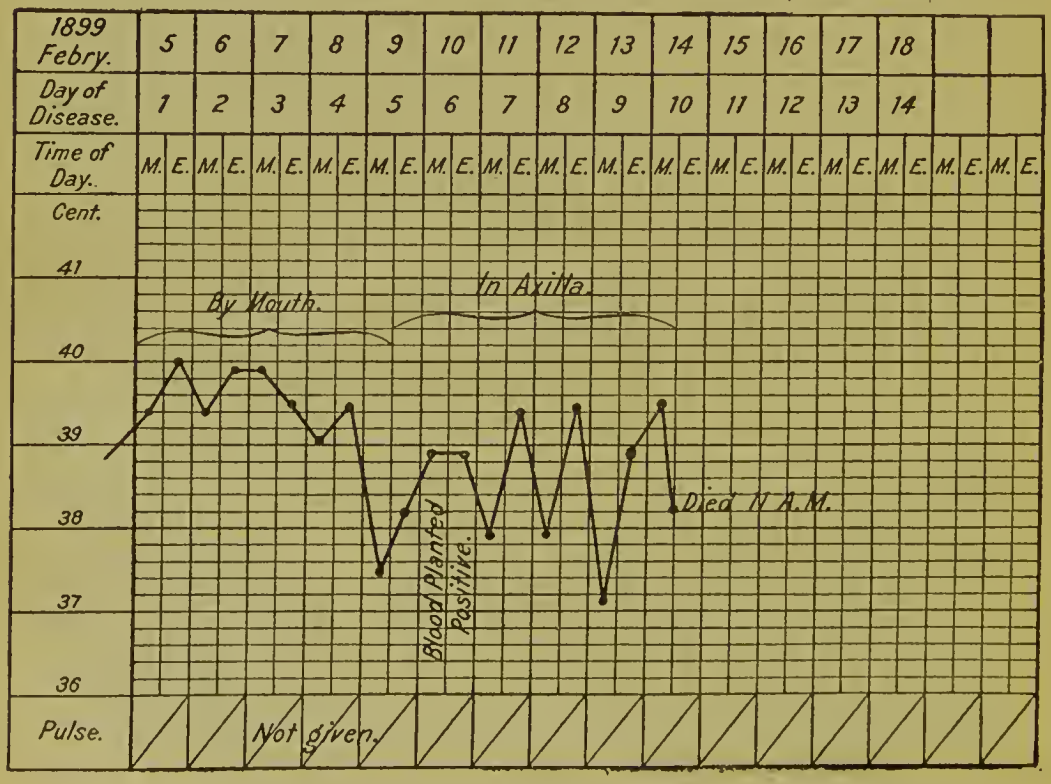
Status presented on the 10th: The face was pale and slightly congested; eyes slightly yellow, pupils dilated; gums pale but puffy; there was no tenderness, save on deep pressure, over stomach and liver; the urine was albuminous: the attendant stated that the stools were yellow

and not black; the skin of body was tinted yellow; there was marked subsultus and an incipient delirium; temperature, 39°; pulse, 82.

When invited to give our opinion of the case, we gave a diagnosis of yellow fever of a serious type, and prognosed death within forty-eight hours. At this visit blood was taken from the ear tip (on the sixth day of the disease), and when plated gave us numerous colonies of b. icteroides; also of two other organisms, one a colon. We offered this organism in evidence as diagnostic on the 12th. Death ensued on the 14th at 11 a. m.

Necropsy three hours later, conducted by an acting assistant surgeon, United States Army: Rigor mortis marked; body yellowish, with considerable hypostasis in dependent portions of trunk; eyes tinted yellow; gums pale; section showed dry tissues; vessels of mesentery much engorged; liver congested, mottled yellow; gall bladder contained some bile; spleen was immense and firm to the touch, and, on incision, the result of preceding malaria; kidneys swollen, capsules non-adherent. Heart: Left contracted, right full of fluid blood. Stomach: External vessels engorged, mucosa swollen and eroded at points, submucosa ecchymotic and stained posteriorly and along the greater curvature; no free hemorrhage; no black vomit. The duodenum was intensely injected; the submucosa deeply stained from extravasations; no free hemorrhage. The entire small intestine was in this state of ecchymotic extravasation and stained submucosa; but at not one point was there an appearance of glandular enlargement, either solitary or agminate. In the neighborhood of the valve the congestion was less than in the higher portions of the organ. At the ileo-cæcal valve, in the mesentery, there were one or two mesenteric glands slightly enlarged. The appendix was not examined. The question of free hemorrhage along this long stretch of congestion was, in our opinion, undetermined. Cultures from all fluids and organs. In twenty-four hours the spleen gave a pure culture of b. icteroides, and the blood a culture but slightly contaminated. Other organs and fluids the same. There was observed no bac. typhosus.

[Case No. 7.—Patrick W. Smith; age, 28 years; disease, yellow fever.]



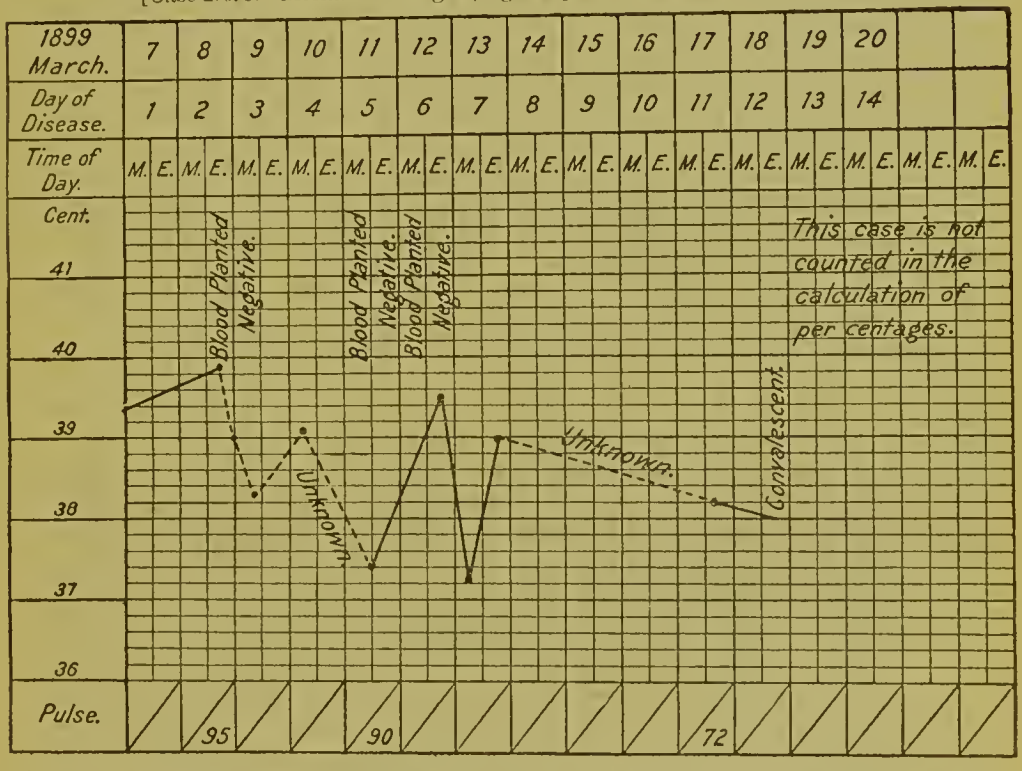
This case was a typical one of extensive invasion of the blood by the organism, a veritable septicæmia, however it started. It resembled closely in this respect the eighth case of Dr. Sanarelli, in which the bacillus icteroides existed in almost pure culture.

On the 12th, from the blood taken, a serum test was made in the dilution of 1 to 20, with a bouillon culture of b. icteroides, and there was not the slightest influence of either motion or of agglutination in three hours. Nor could we get a reaction at any time. This organism we have used very extensively in further work on animals, the resulting necropsies always showing the most characteristic changes in the liver, stomach, and intestines.

Case No. 8.—Michael Lannigan; aged 30 years; a native of the United States; was seen at the Hospital de Nuestra Señora de las Mercedes.

There was a history of nonimmunism; of laboring on the Havana docks, last at the pier San José (a badly infected locality); he became sick on March 7, 1899, a. m., with chill, headache and backache, nausea and vomiting; not much muscular pain. The chill, he says, was followed by fever and "a sweat." Admitted to hospital on 8th. The urine was albuminous; the bowel constipated. He was seen by us on the 11th, 9 a. m.

[Case No. 8.—Michael Lannigan; age, 30 years; disease, yellow fever.]



Status presens: Face dull, with slight passive congestion; eyes yellowish, with dilated capillaries; tongue furred; gums are swollen and retracted from teeth, bleeding on slight pressure; the skin of chest congested upon hand pressure; some pain over epigastrium; spleen normal; urine contains 25 per cent of moist albumen; the stools (one) black and very offensive; no black vomit; temperature, 37.4°; pulse, 90. Took blood from ear tip (fourth day of disease). On the 12th we were informed by telephone that this man was again very ill; a temperature of 39.5°; the albumen increased, as also the nausea and vomiting. Again seen by us, and we again took blood from the ear tip (fifth day of disease), and also samples of the stools and urine. From

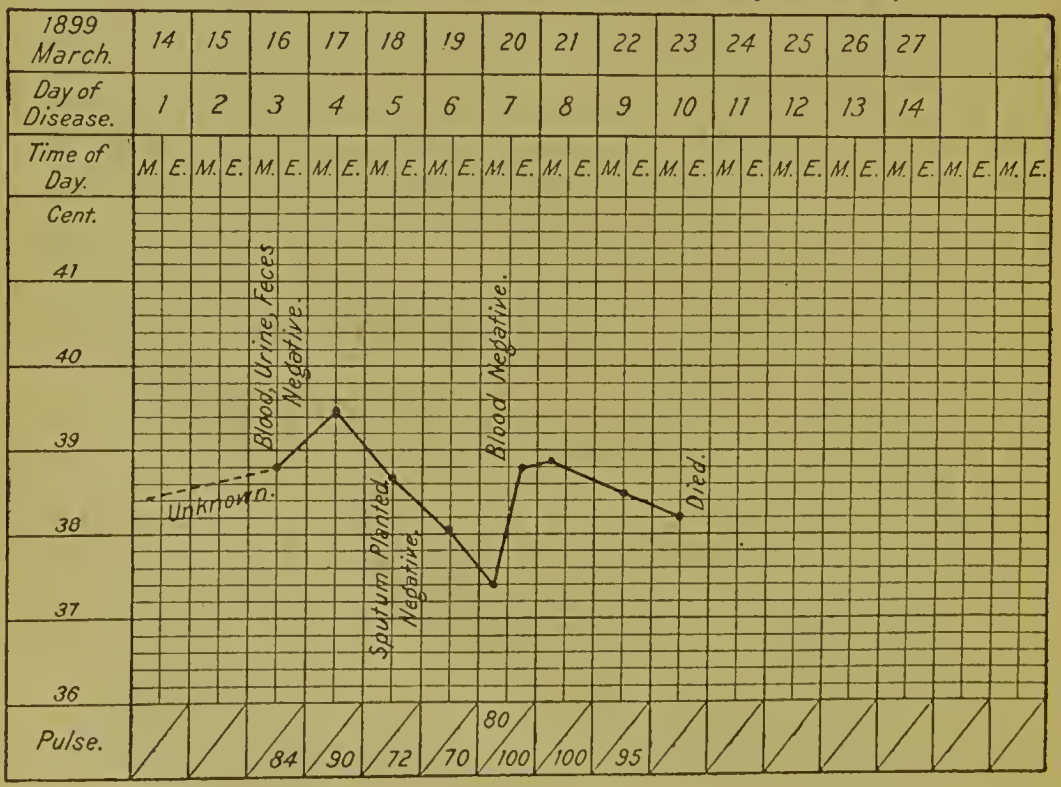
none of these was the bae. ieteroides isolated. From the stool, urine, and blood we isolated that form of b. coli com., called by Sternberg "X" and by Archinard "A and B." On the 13th the man again had an accession of fever to 39° C. Pulse, 100. Quinine administered. This hospital is situated so far from our laboratory that attempts to examine fresh blood were futile. No malarial parasites in stained preparations.

The man again seen on the 17th, was very debilitated, and showed anæmic hallucinations; temperature, 38.3; pulse, 72; marked icterus; urine still albuminous; herpes labialis. Blood taken in bulbs, also urine and fæces, from which cultures were made. No organism save the "X" and a proteus. That this may have been yellow fever at first and afterwards had developed also malaria is most probable, but it remains in doubt. When we first saw him the facies and other symptoms pointed to yellow fever, for at this visit the temperature record was 37.4°., the *first* intermission. After this there were daily intermissions. From the extreme anæmia and debility convalescence was very tardy.

Case No. 9.—Manuel Corujo, a native of Spain; aged 20 years; resident in Havana two months, at Casa Blanca, where he was taken ill on the 14th of March at night, with headache, pain in back and limbs, nausea and vomiting. Entered Hospital de Mercedes on the 16th, with much pain in back and head; the urine slightly albuminous; temperature 39.5; pulse, 75.

On the 18th we saw him for the first time. The skin was icteroid and congested; urine contained 25 per cent of moist albumen. Blood, fæces, and urine taken and planted. On the 19th the albumen was 10 per cent; the skin icteroid; conjunctivæ icteroid; eyes injected; skin still very congested. On the 20th, a. m., blood again taken and planted. On 21st he was semidelirious, there was subsultus tendinum; the albumen was 18 per cent. On 22d in stupor; almost an anuria. On the 23d at midday he died, comatose.

[Case No. 9.—Manuel Corujo; age, 20 years; disease, yellow fever.]



Necropsy (two hours post-mortem).—Body of young adult male; no distinctive marks; rigidity present; skin of body yellow in tint; eyes yellow, pupils dilated; hypostasis of buttocks and loins. Section showed the tissues dry; mesenteric vessels engorged; peritoneal fluid yellow; liver a lemon-yellow, from fatty change; gall bladder distended by a large clot of blood (hematocele); spleen normal in size, dark slate color; kidneys congested, and when incised gave marked fatty areas about the bases of the pyramids of Malpighii; the diaphragmatic pleurae ecchymotic; pericardium ecchymotic; pericardial fluid distinctly yellow; the right heart full of dark blood; left ventricle contracted; the vasa vasorum of the great vessels beautifully distended; stomach contained 500 c. c. "black vomit;" mucosa eroded, stained, and ecchymotic; duodenum the same; bladder contained but little urine, highly albuminous; cultures from all sources; the bac. icteroides was isolated from the blood, but could not be found in the urine nor feces; it was present also in the spleen. From the urine and feces the bac. "X" was readily isolated.

Changes in the liver were as follows: Moist liver, 10 grams; dried liver, 3.22 grams; residue, 1.6 grams; fat, 1.62 grams, or 16.2 per cent of fat in fresh liver and 50 per cent of fat in dried liver.

Case No. 10.—Adolfo Montero; aged 27 years; a native of Spain; residence in Cuba just seventeen days, at "la Casa Blanca." Was taken sick March 19, in the evening, and when admitted to Mercedes Hospital he stated that he had been sick two days from headache and fever.

On the 22d we saw him. Blood was taken from the ear tip and planted. Temperature, 36.8°; pulse, 68; much nausea and vomiting; stomach tender to pressure; spleen normal; liver tender; gums swollen and congested; tongue with red edges; urine dark (hemoglobin), and contained 75 per cent of moist albumen. There was no daily record of temperatures in this ward of the hospital, and all that we could learn was that the temperature had ranged *high* with a *low* pulse rate, although the man disclaims any fever and affects to be much better than he really is. He is a fisherman, and states that a deeply congested and eruptive state of the face, neck, and arms was due to the sun. Diagnosis, yellow fever.

On 23d, temperature, 37°; pulse, 82 and good. He desires food; urine less albuminous and clear; eyes yellow and skin tinted. The bac. icteroides was found in this blood in abundance, together with a variety of b. coli com. Urine gave an "X" bacillus. Convalescence was very slow in this case, the eyes deepening in tint, as also the skin.

Case No. 11.—Captain Williams; British ship *North Anglia;* aged about 35 years; a native of England; was taken sick at 7 p. m. March 5, 1899.

His physician informed us that there had been pain in head and back, with chill and fever to 39.6°.; the pulse not notably low.

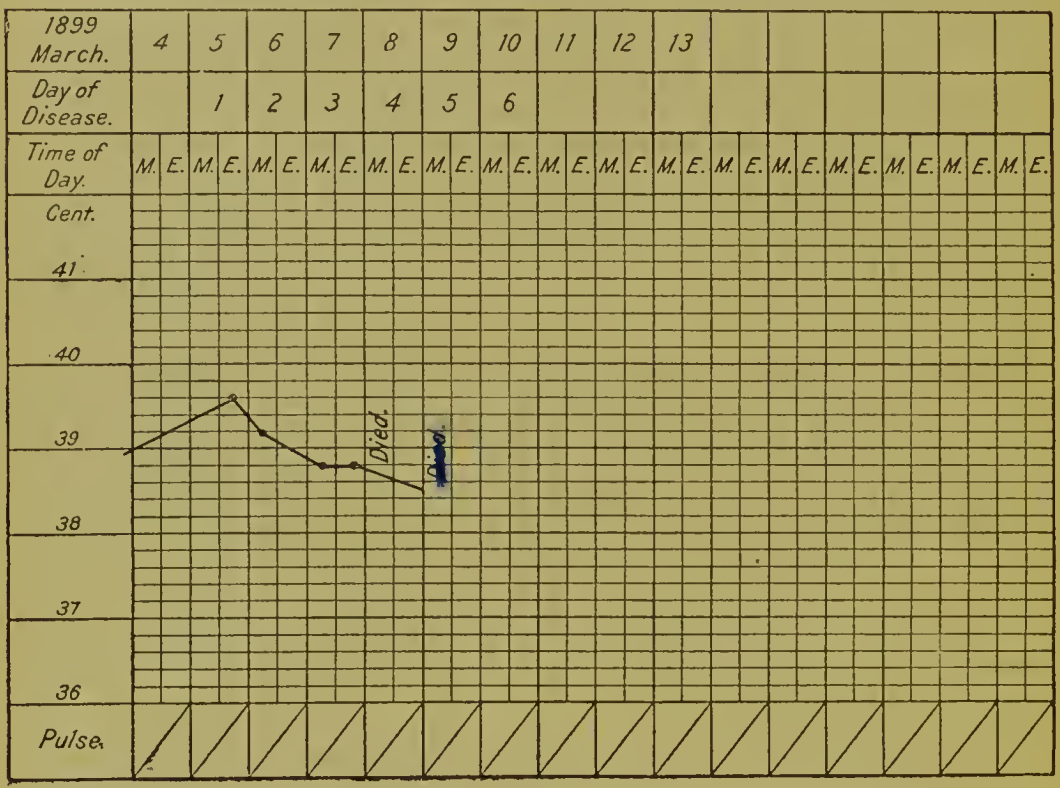
On 6th, temperature 39.2°, nausea and vomiting; on 7th, temperature 38.8°; nausea and vomiting; great congestion of skin of face and neck; vomited dark-looking material. Died at 12 midnight of the 7th; ill just fifty-three hours.

There was no suspicion of yellow fever in this case until the morning of the 8th, when the steward of the ship was reported "very sick." We were notified and held necropsy on the captain at 2 p. m., 8th.

Necropsy.—Body of well-formed adult male; rigidity present and extensive stasis of face, neck, and trunk; eyes, yellow; pupils, dilated; skin, yellow; tissues, dry on section; vessels of the mesentery, dilated; peritoneal fluid yellow; intestines, dry on surface and sticky; *odor*

intense; liver, a fine boxwood color from fatty change; gall bladder contains some 20 c. c. bile; spleen, normal in size, slate-colored; kidneys congested and show, on section, fatty change at the bases of the pyramids. Left heart contracted, right dilated with fluid blood; vasa vasorum of great vessels dilated; stomach contains 500 c. c. black vomit; mucosa ecchymotic; submucosa is stained a deep port-wine color and there is extravasation; the duodenum presents the same appearance; bladder contains a little urine, highly albuminous; lungs not carefully examined; ecchymoses and an infarct noted. Cultures from heart blood, liver, spleen, kidneys, peritoneal, and pericardial fluids, urine, feces, and gall bladder, and contents of stomach.

[Case No. 11.—Captain Williams; age, 35 years; disease, yellow fever.]



This was thought to be a case par excellence for bacteriologic investigation, but we were much disappointed. From the blood tubes taken we (Dr. Geddings) found *one colony* of b. icteroides in a plate from a bouillon tube, the spleen and liver gave a contamination of colon; the fluids and bile were sterile. The black vomit gave a beautiful bacillus "X," presenting the peculiarity, that Sternberg found, of marked motility when *first* isolated, but of *quickly* losing this *permanently*, not even its passage through animals restoring it. Later, through a large rat, to which we fed one of these "X" cultures, which we suspected of containing b. icteroides we (Dr. Wasdin) obtained b. icteroides from its *trachea.*[1]

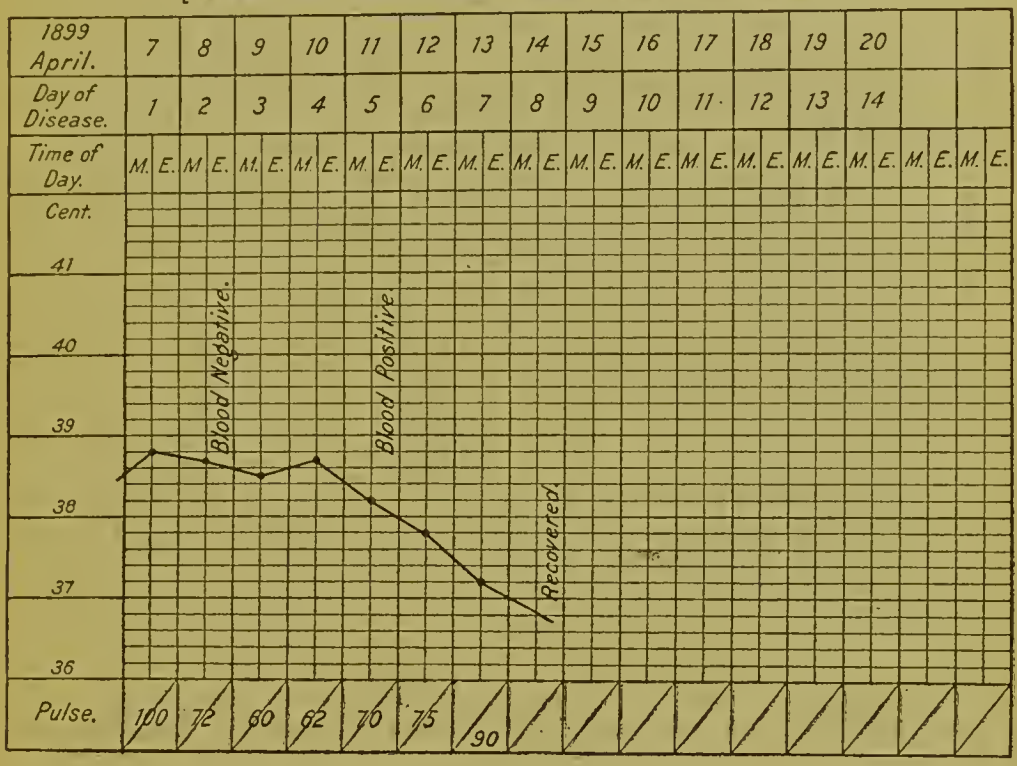
Case No. 12.—Everette, steward on the *North Anglia;* about 35 years of age; English; was taken sick the morning of the 7th of April with

[1] This rat was caged in the closest proximity to several cages in which mice were being exposed to bac. icteroides, and since there is possibility of this rat having caught the infection from these cages, we have not used the result of the isolation of the bacillus in any percentage calculations.—(Wasdin.)

chill and pains in the back and head and limbs; nausea and vomiting. On the 8th, at 2 p. m., we saw him.

Status presens: The face is suffused, with some swelling about eyes and lips; conjunctivæ injected and yellow; eyes smoky; gums bleed on slight pressure; skin generally congested and yellow; urine scant and contains 25 per cent of moist albumen; we collected blood from the ear tip, in Sternberg bulbs.

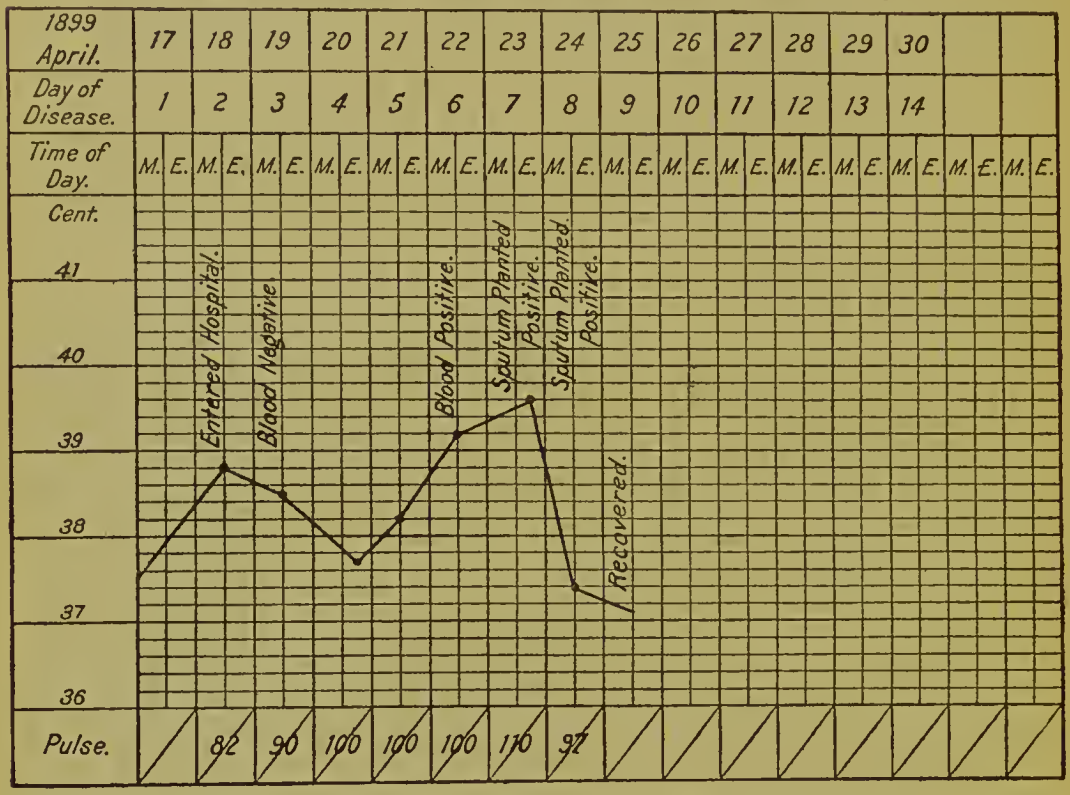
[Case No. 12.—Everette; age, 35 years; disease, yellow fever.]



On the 10th there was bloody expectoration with mucous râles in both subscapular areas, and some evidence of a lobular pneumonia. On the 11th again took blood and planted it; urine, 1.014, alkaline, with 25 per cent moist albumen. On the 12th he still expectorated bloody mucus. On the 13th he entered upon a tardy convalescence. A feature of this case were the lung symptoms, which gave rise to some alarm. His physician states that a bubbling fine râle was present when he first saw him, and that the bloody mucus was very free for several days. The blood serum of this man, diluted 1–20 and 1–10, gave absolutely no reaction in one hour with b. icteroides (G.). The bacillus icteroides was isolated from his blood taken on the 11th, but not from that on the 8th. A consultation of several physicians pronounced it yellow fever.

Case No. 13.—Kauna Sesein; aged 24 years; a native of Turkey; a resident of Havana two months. Seen at Hospital de los Angeles, April 19, 1899.

[Case No. 13.—Kauna Sesein; age, 24 years; disease, yellow fever.]



History.—She was taken sick on the morning of the 17th of April; no chill, but high fever; pain in head and back and limbs; great nausea and tenderness of epigastrium. When admitted, on the 18th, urine contained 20 per cent of moist albumen. On 19th she was seen by us, and blood taken and planted; the gums and skin were swollen and congested still; urine albuminous. We again took blood on the 22d. On 23d and 24th we obtained sputum, which was planted. This case was poorly pronounced, yet the blood taken on the fourth day gave colonies of the b. icteroides; also from the sputum there was obtained the bac. icteroides in pure culture.

Case No. 14.—Pedro Quintela Macias; aged 30; from Spain; came to Cuba on January 29, 1899; residence at "la Casa Blanca," a notably infected village. Diagnosis of yellow fever.

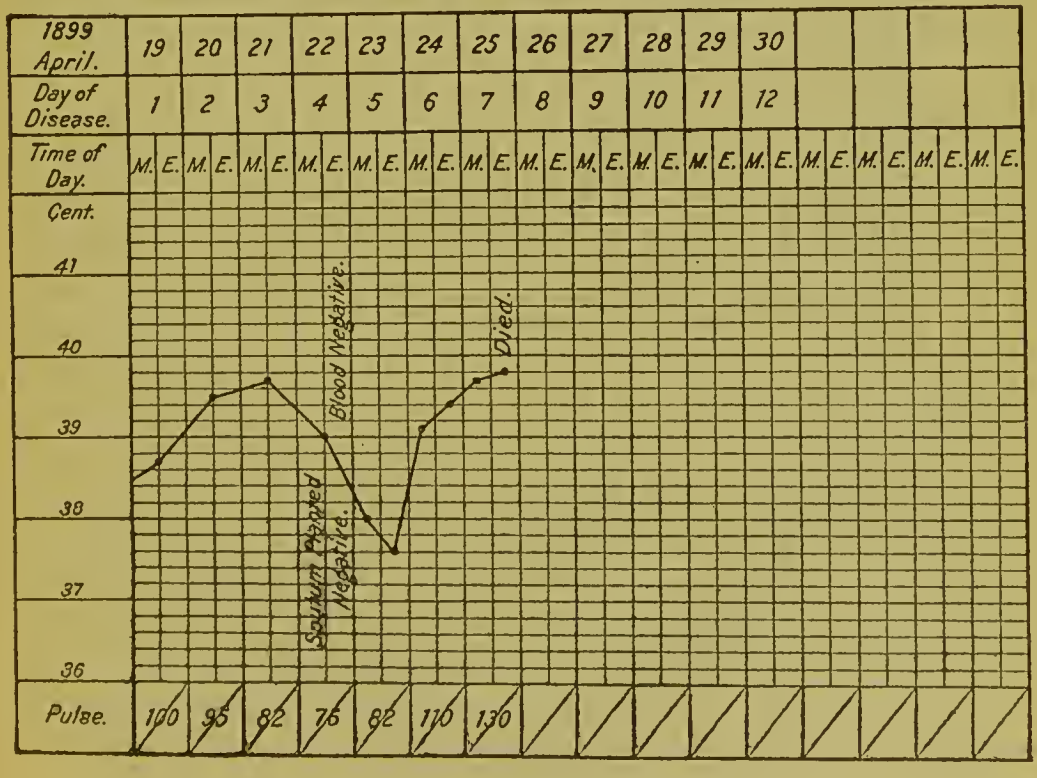
No preceding illness. He was taken sick on Tuesday, 18th, at night, with a chill and high fever; severe pains in head and back and limbs; severe pain in the stomach with nausea, but no vomiting. He was admitted to "Hospital de los Angeles" on the 19th of April, 1899. On 22d he was seen by us. He then presented a congested face; pupils partially dilated; conjunctivæ yellow; vessels dilated; skin of body decidedly icteroid; some diarrhea; scanty urine, contained 25 per cent of moist albumen; specific gravity, 1.013; blood taken from ear tip and planted. There was some bronchitis; the sputum was distinctly blood-tinged and purulent. This was taken, and also the blood, and planted. On April 25 he died at 4 p. m.

Necropsy (two hours later).—Body of well-developed male adult; no distinguishing marks; rigor fairly established; skin yellow, with much

hypostasis; eyes yellow and pupils dilated. On section the tissues appear dry; the vessels of the mesentery engorged; the contents of the abdomen of a rich yellow color; the liver of a deep yellow color, with congestive mottlings; the spleen slightly enlarged, quite firm; kidneys congested; bladder empty; gall bladder full of green bile (had not vomited much); the left heart firmly contracted; right distended with fluid blood; the vasa vasorum of the great vessels distended; pericardium ecchymotic on visceral layer; pericardial fluid yellow; the stomach and intestine hemorrhagic; the *lungs* especially studied; the trachea contained bloody sputum, its mucosa was injected; the bronchi were also full of stained mucus of a purulent character, their mucosa ecchymotic and swollen; the bronchioles were full of frothy, stained, purulent mucus. The right lung gave most change; the visceral pleura was distended at many points on the lower lobes with emphysematous dilations of the superficial air cells; it was ecchymotic, some extravasations being as large as the finger nail. On incision there was evidence of emphysema from the obstruction in the tubules; the vessels were engorged and the catarrhal secretion free, but not to the extent of a catarrhal pneumonia, the lung floating high in the water bath. We were forcibly reminded of the lung in "la grippe," but there was not that peculiar loss of elasticity of the lung framework which we have found in cases which have died from "grippe." The left lung presented the same picture, only less pronounced. The pleuræ were ecchymotic in both layers; the pleural fluid yellow. Cultures were made from all specific organs and fluids, from which the b. icteroides was *not* isolated. From the sputum we isolated the bac. "X," very motile, and the bacillus of sputum septicæmia.

From this case, we are informed by Surgeon-Major Meacham, U. S. V., that the bac. icteroides was isolated by him from the blood of the lung.

[Case No. 14.—Pedro Quintela Macias; age, 30 years; disease, yellow fever.]



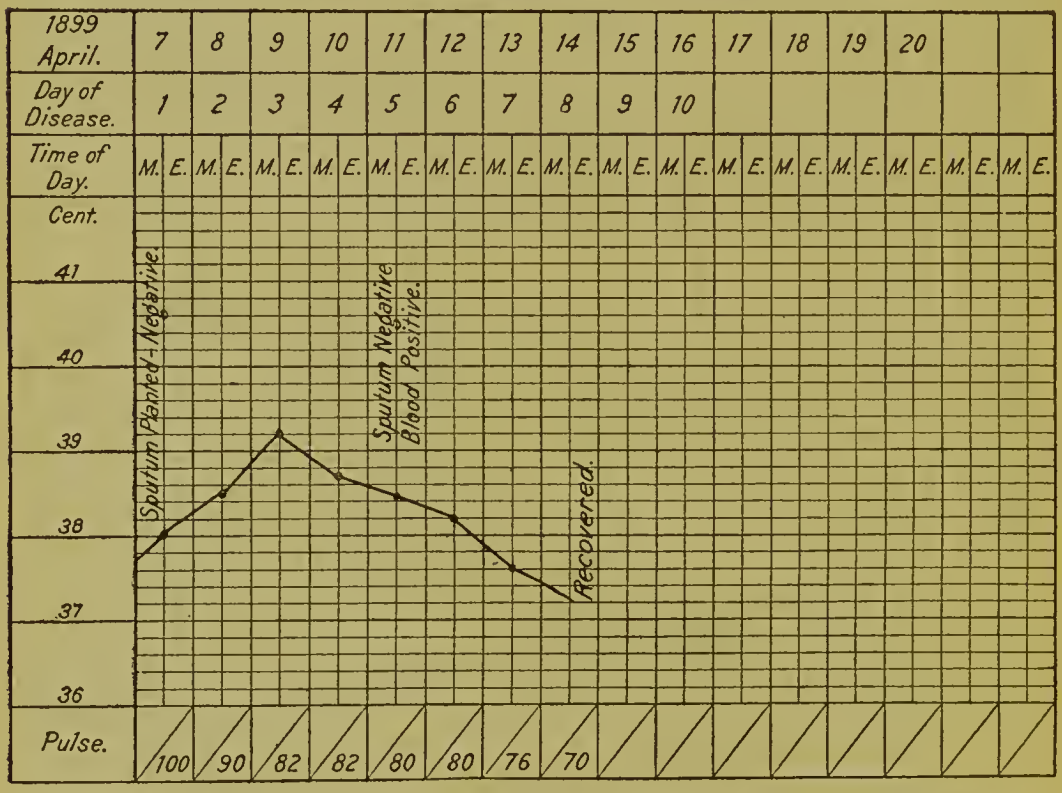
Case No. 15.—An unknown man, evidently of Spanish origin, and aged about 40 years; was seen at Los Angeles Hospital on the 10th of March, 1899.

There was no history whatever. He was unconscious when admitted from la Casa Blanca. When seen the man was moribund; unconscious; pupils dilated; face flushed; dry, bloody sordes about the teeth; tongue dry; breath fetid; gums swollen; skin icteric; urine scant and voided in bed; pain on pressure over liver and stomach evidenced by facial expression; delirium of low type; blood taken from ear tip and planted. Though clinical evidences in the case were not complete, nevertheless we made the diagnosis of yellow fever. The blood was taken and planted in bouillon, and from this plated, when it gave numerous and typical colonies of b. icteroides. Death ensued at 10 p. m. of the 10th. No necropsy was made.

Case No. 16.—Joachim Rodriguez; 19 years of age; one and a half months from Spain; in perfectly good health until May 7; residence on Aguacate street, a bad locality in Havana. During his stay in the city he has been employed upon the repairs and cleaning of the city sewers.

On the 7th of May he was taken sick with violent pains in his head and back. He stated on entry to the Los Angeles Hospital that he had had

[Case No. 16.—Joachim Rodriguez; age, 19 years; disease, yellow fever.]



"*no* chill nor fever." He presented a flushed face; yellowness of the conjunctivæ; some pain in epigastrium; no nausea nor vomiting; temperature, 38°. There was bronchitis, and bloody sputum which was planted; the urine was albuminous. On the 11th the sputum was less bloody; again taken and planted. On the 13th there was convalescence. The blood was taken on 11th, and from it the organism isolated; b. ictoroides. But the sputum did not give it.

Case No. 17.—B. H. Powell; aged 30 years; American; recently arrived in Cuba; fell ill on the night of March 19 in the house adjoining the one in which Doane had died.

He gave a history to Dr. Menocal, who saw him for us, of headache and pain in left hypochondrium and abdomen. There was no icterus; no change in gums; no congestion of face nor skin of chest; no vomiting, and little nausea; there was but little fever; the temperature, 37.9°; pulse, 84; only a trace of albumen in the urine. Dr. Menocal did not concur in the diagnosis of yellow fever of the attending Cuban physician. No further information could be obtained. The blood taken on the 21st of March gave no organism of any interest. No sputum was obtained.

Case No. 18.—Gomez Garcia; aged 25 years; a Spaniard living at Casa Blanca, was admitted to the Hospital de Los Angeles at 8 p. m. of the 14th of March, 1899, quite ill and unconscious.

No antecedent history. We saw him on the 15th.

Status presens: He was semiconscious, dull, and stupid; defecation and urination uncontrolled; clothing was stained a brilliant saffron hue; it also showed signs of vomiting in dark green stains, with "fly-wing" threads; urine could not be gathered; skin cold; temperature, 36.8°; pulse, 120; eyes a little yellow; gums swollen and blue; tongue broad and furred and indented. Altogether it was thought to be a case of malarial intoxication, which differed from the diagnosis of the attendant of "yellow fever." Blood drawn from ear tip clots quickly in the bulbs; the serum showing a straw color; there was only a slight reaction of Widal, in 1–40, in half an hour.

On the 16th the attending physician voluntarily called at our laboratory to report that he had found the case to be "*not* one of yellow fever." The sputum was not obtained; the blood gave no organism of any interest.

Case No. 19.—Private Perkins, of the 202d New York Volunteers, stationed at Guanajay, Cuba, was seen by us on the 11th of February, 1899, in regimental hospital, the surgeon being absent.

There was the history of a twelve (preceding) days' illness and of some light malarial attacks during January; also of exposure to the unclean places in the seacoast town of Mariel and of exposure to the sun. He presented a bright, alert facies; a fine twitching of the muscles about the mouth, and other signs of subsultus; tongue furred and red-bordered; gums normal; no great nausea nor vomiting, only a little greenish fluid; no skin congestion; some pain in region of ileo-caecal valve; urine held a trace of albumen for several days; there were no rose spots; no epistaxis, and but little inclination for food. Blood taken in Sternberg bulbs.

This case had been diagnosed yellow fever, from which one of this regiment was said to have died a few days before. We could not concur in the diagnosis.

The blood gave fine growths of a proteus rod, liquefying gelatin on the fourth day, coagulating milk on the fourth day, and without reaction in the sugar bouillons; also a staphylococcus aureus.

Case No. 20.—A. L. Atwood; army employee; aged about 30 years; was seen at the hospital "de los Angeles" on February 10, 1899, having entered on the 7th.

His skin was yellowish, and eyes injected (he had been unconscious on entry); pupils dilated; history (statement medical interne) of one attack of black vomit, at least of blood, and of several black stools; there was 40 per cent of albumen in the urine, which was scant; there was the statement of a temperature to 38.5°; this was then 37.6°; pulse, 84; blood taken from ear tip and planted.

On the 15th, there having arisen much discussion as to the diagnosis

in this case, one consultant having diagnosed "gastric ulcer" and the other "yellow fever," we again saw the man. He presented undoubtedly yellow eyes; skin, the same; gums bled easily; the urine contained 20 ctgs. of dry albumen to the liter. While we at first, taking the statements of the attendants of this hospital in lieu of better records, of which none are kept of temperature and pulse, thought the case like yellow fever, we did not concur in the diagnosis, but could not concur in that of "gastric ulcer." The b. icteroides was *not* in his blood; the sputum was not examined.

He told us afterwards that he had had similar attacks before, and they had been styled "epileptic seizures," and always had resulted in unconsciousness and vomiting of blood; however, he could not recall any mention by his former physicians of albuminuria, a rather constant factor in hysteria and epilepsy, but of short duration, whereas this (40 per cent moist, and 20 etgs. dry albumen to liter) lasted for several days or a week. The organism isolated from this blood was a staphylococcus, a tetrad and evidently a contamination, innocuous to animals. His blood serum, diluted 1–20, gave with b. icteroides a prompt reaction in the elumping and agglutination within half an hour.

Case No. 21.—Private Bradshaw, B Company, Eighth Infantry, United States Army; aged about 30; native Amerieau; was admitted to regimental hospital on the 20th of February, 1899.

He has a history of Santiago fever. The spleen is permanently enlarged; the bowel inclined to a diarrhea of pea-soup character. When seen on 23d the temperature chart showed morning remissions of an irregular type; there was a distended abdomen; pain over the valvular region; rose spots over abdomen; a red-bordered tongue, dry and fissured; a tendency to facial subsultus; urine with 5 per cent of moist albumen; there was some congestion of skin, as shown by finger pressure; facial expression listless; eyes not yellow. From the experience of the case of Patrick Smith, in the same regiment, this case was very closely watched by the attendants, but at this date we at once diagnosed a typhoid fever in its second week. Blood extracted from the ear tip; the urine and stool taken, and cultures made from them. From urine a proteus, a colon, and a leptothrix were isolated; no b. typhosus; from the blood there was isolated an actively translatory rod, not producing fermentation in lactose; coagulating milk on the seventh day; not liquefying gelatin; and since there was no indol production, it was placed as coli communis of rare type; no b. typhosus from feces. Injected into a pig, this organism, in 1 c. c. dose, reduced the animal 125 grams in twenty-four hours, and it was intensely ill, but slowly recovered.

Case No. 22.—A case of yellow fever seen by Acting Assistant Surgeon Menocal, M. H. S., who concurred in the diagnosis, extracted blood from the ear tip in bulbs, and transmitted them to us at Key West, Fla. These bulbs were kept from this time—April, 1898—until December of that year, when they were unsealed and their contents planted, from which there resulted mixed cultures of b. icteroides and b. coli communis, a surprisingly lengthy symbiosis in view of the declaration of Sanarelli that there exists so marked an antagonism between these organisms.

Of these foregoing 22 cases, the diagnosis was concurred in by one of us (Dr. Wasdin) in fourteen instances; in seven instances there was absolute nonconcurrence; in one instance, the case of Lannigan, No. 8, the diagnosis of yellow fever seemed correct at first, but was founded upon unreliable statements which seemed corroborated by the condition pre-

sented to us. The marked malarial seizures, however, soon questioned this diagnosis. In one instance, No. 14, there was no doubt whatever of the diagnosis, yet we did not isolate b. icteroides, although it was found by Dr. Meacham. In thirteen instances we isolated b. icteroides in pure culture.

Therefore, eliminating all reasonably doubtful cases and including the organism found by Dr. Meacham, we have the even percentage of isolations of the organism, the b. icteroides.

In the blood of yellow-fever cases extracted during life we have found bacillus icteroides in 13 of the 14 cases, with 1 negative—a per cent of 92.85. (In this negative case Dr. Meacham obtained it post-mortem.) From our necropsies we have secured it in 85.7 per cent, which includes the cases from which material was secured in New Orleans in 1897.

We have invariably demanded the completion of every requirement as advanced by Sanarelli in the identification of this organism culturally, beside which each organism has been tested as to its pathogenicity to animals, and we find that the germ exercises the same influence upon the lower animals, whatever its source.

The cultural and physiological characteristics of the bacillus icteroides are as follows:

Form: Small, fine, slender rods, 1.5 to 2 μ long, and three to four times as long as broad. Occurs singly or in pairs; short threads from cultures in bouillon, but never from solid media. Ends rounded, sometimes almost a cocco-bacillus in shape.

Motility: Very motile, with great directness of movement and very free translation across field of microscope. Flagella have been demonstrated.

Sporulation: Has *not* been observed.

Staining: Readily stained with all ordinary basic anilin dyes. Decolorized by Gram's method.

Aerobiosis: Is a facultative anaerobe.

Temperature: Grows best at 37°.

Agar-agar: On inclined agar at 37°–39° isolate colonies are thin, flat, grayish, circular. In two to three days colonies will present a peculiar appearance; a thick opalescent ring surrounding a flat, thin, transparent area. Outer border becomes transparent and original colony shows as opaque, embedded body. Agar streak culture—like typhosus—a thin, transparent growth.

Gelatin: Does not liquify. Gelatin (10 per cent) plates, 16° to 18°. characteristic colonies. Deep colonies are perfectly circular, sharp bordered, waxy; later dark, sometimes perfectly black; showing, possibly, in center slight radiating lines. Atypical colonies do not become black, are lobular, or surrounded by fibrils. Surface colonies resemble droplets of boiled starch or mucus; thick, convex, perfectly circular, or sometimes kidney-shaped. An opaque yellow-white nucleus can be seen at or near the center or at the hilum in the kidney-shaped colonies. It is opaque, finely granular, border sharp and smooth. Nucleus round, more often hat-shaped; in kidney-shaped colonies the crown of the hat is turned toward or projects into the hilum.

Milk: Is not coagulated.

Indol: Not formed, or very slightly; no production of nitrites.

Gas production: In 2 per cent exhausted lactose bouillon, no gas; saccharose bouillon, no gas; glucose bouillon, gas, $\frac{1}{H}\ \frac{2}{CO_2}$.

Bouillon: There is a diffuse growth without the presence of floccules, pellicle, or appreciable deposit.

Potato: There is an invisible moist growth.

Acid production: Slight in glucose; saccharose and lactose neutral reaction.

Pathogenesis: Pathogenic to rabbits, guinea pigs, rats, mice, dogs, cats, and monkeys.

It is unnecessary to remark upon these now well known attributes of the bac. icteroides, since the organism in pure culture has remained unchanged from the time of its discovery, and has proved to be the same in all respects wherever isolated. It may be mentioned here that in our earlier work with the organism, and owing to a misapprehension of the possible changes in our sugar media under certain conditions, we stated that there was no evolution of gas by its growth in glucose media, but later it was found that this observation was incorrect, and depended upon the facts given in our letter to the bureau upon this subject.[1] In this connection it may be stated that the varying reports from different observers as to the influence of this organism upon lactose depend upon changes produced in the molecular grouping of the constituents of this sugar by the application of too great heat in the preparation of its media. Hence if there is present in such media fermentation with gas production there is evidence of an inversion of a portion of the lactose by heat prior to the inoculation of the medium with this organism, which readily ferments the glucose thus formed, but has no power of inversion. The same may be said of saccharose.

Our attention has been called to a very striking similarity between the cultural and physiologic characteristics of b. icteroides and b. choleræ suis.[2] Our own observations lead us to state that, while there are singular resemblances, the b. choleræ suis differs from b. icteroides in presenting a grosser expansion, more distinctly slimy or ropy, a somewhat dirty appearance, on agar-agar slants, than does b. icteroides of the same age, which remains permanently clear and transparent. In bouillon cultures there is with icteroides no disposition to form ropy deposits, which are seen with choleræ suis. Also, we have not noticed any disposition on the part of b. choleræ suis to form the seal-like colonies, said by Sanarelli to be distinctive of his organism. Moreover, b. choleræ suis seems to have the property,[3] not possessed by icteroides, of forming sulphur compounds (H_2S), even in the absence of albuminoid material, as in the solution of Dunham, which can be demonstrated by appropriate reagents. Other differences between these organisms will be mentioned in another place. As stated above, we have made in each case every one of the demands before assenting to the fact that the organism isolated was the bac. icteroides, and in no case did we allow a cultural appearance, or several of them, to influence us in the adoption of the organism as the true icteroides until all requirements were fulfilled. We believe that serious error has resulted in the work of others from a neglect of this precaution.

ANIMALS ARE SUSCEPTIBLE TO THE INFECTION OF YELLOW FEVER.

The accepted methods of subjecting animals to the artificial influence of pathogenic organisms is in most cases above criticism—that is to say, artificial inoculations resulting in well-marked pathologic lesions in the bodies of animals *similar* to those found in such diseases in man must be considered as a fulfillment of the third postulate of Koch, even

[1] Letter to bureau March 3, 1899.
[2] Reed and Carroll, Med. News, May, 1899.
[3] Observation by Geddings.

also of the fourth. But the same or similar pathologic lesions may be produced by different organisms introduced after these artificial methods, as the subcutaneous, intravenous, or intraperitoneal injections, and although there may be a difference in the quantity of the anatomic changes, yet there is the same quality of change.

Thus with yellow fever practically the same lesions are produced in animals inoculated with the virulent varieties of the bac. coli communis as with that of yellow fever; and more recently the same observation attaches to bac. cholerae suis in mice and guinea pigs. We repeat that in animals which succumb to these artificial infections we find a marked similarity of appearance at necropsy, the difference being one of degree and not of kind. Moreover, we find that this observation applies to the results from the exhibition of the toxins of these different organisms.

We have had in our experience animal necropsies from the bacillus "X" of Sternberg, and from that of Havelberg, which could not have been told from those from bacillus icteroides. Especially has this been so in rabbits, whose livers do not become extremely steatotic. Therefore your commission could not accept the conclusion that Sanarelli had proved his position satisfactorily, and this led us to institute a series of experiments with susceptible animals, which we feel has resulted in the demonstration that the bacillus icteroides is the natural cause of yellow fever, and that those organisms which may produce similar changes in the organs when introduced into the tissues artificially *can not* produce the *acute infection* of yellow fever. Sternberg has contended that his bacillus "X" is identical with the bacillus icteroides of Sanarelli,[1] and the inference to be drawn from this contention that therefore bacillus "X" may be the cause of yellow fever is not surprising, since we have found bacillus "X" in a large number of our cases associated with icteroides, and "X" is capable of producing changes in the organs strikingly like those produced by yellow fever.

A case is recorded in which the colon bacillus has produced death in man, with anatomic lesions indistinguishable from those of yellow fever.[2] In this case the infection was artificial, since the b. coli communis has no natural infectiveness for man or animals, and was evidently derived from appendiceal abscess.

Therefore in these animal experiments made by "natural" infection the bacillus "X" and that of Havelberg, the two most prominent candidates for distinction, have been dismissed from further consideration after their failure to infect white mice, these rodents being considered the most susceptible animals to the acute infections.

On March 23, 1899, 2 p. m., we submitted 10 white mice, two in each cage, as follows, viz:

Cage No. 1.—Fed on bac. icteroides from Case No. 18 New Orleans 1 c. c. bouillon culture twenty-four hours old. Mouse C died on April 5 at 6 a. m., or twelve days and sixteen hours after exposure to infection.[3] Mouse D died on April 5 at 10 p. m., or thirteen days and eight hours after exposure.

Cage No. 2.—Fed on bac. icteroides from "Smith," twenty-four-hour bouillon culture on food. Mouse E died on April 1 at 5 a. m., nine days and three hours after exposure. Mouse F died on April 2 at 2 p. m., or just ten days after exposure.

Cage No. 3.—Fed on bac. "X" of Sternberg, twenty-four-hour bouillon

[1] Journal Am. Public Health Assn., Oct., 1898, p. 426.
[2] Annual Report M. H. S., 1898, pp. 437, 438.
[3] For necropsic findings on this and following animals see pages —.

culture on food. Mouse G, on April 24 in fine order. Mouse H, on April 24 in fine order. Both continued well thereafter.

Cage No. 4.—Fed on bac. typhosus (Krâl), twenty-four hour bouillon culture on food. Mouse I died April 1 at 5 p. m., or nine days and three hours after exposure. Mouse J was found dead April 16, after it had been transferred by an attendant to cage No. 2, which had not been sterilized. Just how long it had been in this cage could not be learned.

Cage No. 5.—Fed on bac. coli communis from hygienic laboratory, M. H. S.; twenty-four hour bouillon culture on food. Mouse K on April 24 was in fine condition. Mouse L was lost from cage on March 25, 1899.

Thus is seen that those animals subjected to the b. icteroides from two sources, No. 18 New Orleans, and from the case of "Smith" in Havana, all died; the organism more recently from Smith being most virulent. There was one death from typhoid infection; the other mouse died after exposure to b. icteroides, which were found in its blood, but not b. typhosus. Those subjected to the "X" bacillus and to coli communis did not at any time appear ill.

From these experiments it was not possible, since it is not practicable, to follow the health conditions of the rodents very closely, to formulate any idea of the *period* of *incubation*, nor of the duration of the disease, so that on the 20th April we exposed other mice, as follows:

Cage No. 6.—Fed on b. icteroides from "Smith," in bouillon. Mouse K^2: On April 24 we gave this mouse a bichloride bath, a new sterile cage and cotton, and placed with him a new mouse, K^3. K^2 died at 6 a. m., April 27, 1899, or six days and thirteen hours after feeding; left in cage three hours. Mouse K^3 remained well until May 8, when b. icteroides was added to the food. It died May 20, 1899.

In this instance mouse K^2 became infected during the first four days, but entirely failed to infect mouse K^3 or the cage, although left in the cage three hours post-mortem.

Cage No. 7.—Fed on b. icteroides from Smith, which had been scraped from a large plate culture and mixed with sterile lycopodium and dried slowly, and this powder dusted upon bread; the cage kept dry. Mouse L^1: On April 24, as with K^2, this mouse was given a thorough bichloride bath and a clean cage and cotton; also a new mouse, L^2; died at 12 m., April 27, 1899, or in six days and twenty-one hours. Mouse L^2 remained well until May 8, when b. icteroides was added to food; died May 28, 1899.

Again the infected mouse, L^1, failed to infect L^2, but, from the fact that the b. icteroides has been added to the food of L^2 on several occasions since May 8, we are inclined to believe that L^2 became infected and recovered, although, as we have said, it is very difficult to observe symptoms, save those of "extremis" in these small animals.

Cage No. 8.—Fed on bac. icteroides in lycopodium. Mouse M^1: On April 24, as in two above, the mouse was washed in bichloride solution and given a clean cage and cotton and a new mouse, M^2. M^1 was quite sick, but recovered. Mouse M^2 died May 4, at 9 a. m., ten days from time of exposure to the sick, M^1, in the clean cage.

Cage No. 9.—Two white mice were placed on bac. icteroides in their food on March 13, 1899. Mouse A died March 21 at 6 a. m., or seven days and eighteen hours from time of exposure. Mouse B died on March 22 at 6 a. m., or in eight days and eighteen hours from time of exposure.

From these instances it is seen that, with the exception of the mice K^2 and L^1, which were exposed to a cold bath of mercuric chloride

solution 1-3,000 from three to five minutes, and which died in six days and thirteen to twenty-one hours, these animals died in from ten to twelve days. And since the mice exposed on April 20 in the three cages (K^2, L^1, and M^1) all became sick and were washed and changed on the 24th, it must be concluded that infection in mice takes place within the fourth day after exposure, and therefore that the duration of the disease, if uninfluenced (as by the baths), is from six to eight days.

Mouse N was fed upon b. icteroides, for the purpose of obtaining organs rich in virulent germs to be fed to the domestic pigs, on May 8, 12 m.; died on May 17, 8 a. m., or eight days and twenty hours after time of exposure.

These cases show a marked regularity in the influence of this organism upon white mice—that it is not invariably fatal to them, and that the bacillus of typhoid is also infectious naturally to them.

Again, a large white rat was caged and fed upon bac. icteroides from Smith, April 17, at 12 m. April 18, animal eats well; April 19, eats but is very quiet; April 20, eats little and appears ill; April 22, eats and appears better; April 24, eats and is better; April 29, eats well and appears well; apparently infected and recovered.

Also a large brown rat was caged and fed upon a bouillon culture (1. c. c.) in food of an organism styled "Williams's X," which, a note made at the time stated, we believed to be mixed with bac. icteroides. On 18th, eats well; 19th, eats well, appears warm, pants; 20th, eats a little, appears sick, pants; 21st, eats little, appears ill; 22d, eats little, appears ill; 23d, eats a little, appears somewhat better; 24th, eats a little, appears somewhat better; 25th, eats a little, appears somewhat better; 26th, eats a little, appears quieter and pants, much dyspnœa; 27th, eats; died at 12 m. of yellow fever. (See necropsy, page 33.)

From these larger rodents it is seen that the organism of Sanarelli is naturally infectious to them; that the period of incubation is about three days; that one recovered after several days of illness; therefore it is not invariably fatal to them; that in the fatal case the duration of the disease was possibly six days and eighteen hours, the incubation being counted as three, or nine days and eighteen hours from exposure. Also this animal's clinical history recalls vividly the history in human cases.

Other than these experimental mice, there have been observed three cases of natural infection within our general cage—mice "X," "XX," and "XXX." These were first observed either just before death or just after demise, and will be again referred to later on.

NECROPSIES ON ABOVE ANIMALS.

All these animals were necropsied, many of them at once, and all were placed in the refrigerator when found at inconvenient hours. These necropsies are as follows:

Necropsy mouse A.—Died 6 a. m., March 21, 1899. Much congestion of the vessels of the skin; the tissues are dry; the inner surface of the pelt is distinctly yellowish; vessels of the mesentery are engorged; the liver is engorged and apparently fatty; the spleen is enlarged and full of blood; the pleuræ seem normal, yet the lungs are much congested (tubes not examined); heart distended in right side; stomach contains a few spots of free blood; mucosa is ecchymotic and the submucosa stained; the duodenum is ecchymotic; kidneys are congested; the urinary bladder is empty. Cultures from organs and fluids. Liver saved for examination; the teased tissue in osmic acid solution shows much fatty degeneration of the hepatic cells, and the fat, extracted by

trituration with ether from a given amount of liver and collected by evaporation on the surface of a water bath, gave 8.25 per cent of the liver weight in the moist state, and when dried and extracted it gave 29.5 per cent of liver (dry) weight, leaving no possible doubt as to fatty degeneration of a marked type. From the culture tubes we obtained a pure culture of b. icteroides; in this case there is no contamination, and this is made to fulfill all cultural demands according to Sanarelli.

Necropsy of mouse B.—March 22, 1899. Some rigor; vessels of skin congested; there are bloody sordes about mouth; the tissues are dry; the inner surface of the pelt is of a golden-yellow color; the mesenteric vessels are congested; the abdominal contents are distinctly yellow; the liver is congested and yellow from fatty change; the spleen is slightly enlarged; the stomach contains black vomit and duodenum the same; the kidneys are congested; the bladder contains a little urine, *bloody*, which shows much albumen when added to Esbach's picric acid solution; the heart contains much fluid blood; the lungs are congested, the right *not* collapsed fully; no increase of pleural fluid. Cultures from organs and fluids and intestinal contents (bloody). The organs and *urine* contain b. icteroides in pure culture; the tubes from stomach and duodenum show it in mixed culture. Amount of fat, by weight, in the moist liver tissue, 10.2 per cent; in the dried, 50 per cent. This little animal certainly gave a typical necropsy of yellow fever.

Necropsy of mouse C.—Dead from b. icteroides obtained from necropsy No. 18, New Orleans. Vessels of skin congested; inner surface of pelt yellowish; vessels of mesentery engorged; the liver mottled reddish and yellow, very fatty; the spleen slightly enlarged; the kidneys are swollen from congestion; bladder contains a little albuminous urine; heart is full of dark blood, left ventricle seems empty; lungs congested; pleuræ seem normal; the mucosa of stomach is bathed in yellowish mucus and contains several extravasations, but there is no *free* hemorrhage; the intestinal mucosa is congested and stained, and shows free hemorrhage at two points; cultures from blood and organs and intestinal content. Moist liver, 0.895 grams; dried liver, 0.345 grams; residue, 0.198 grams; fat, 0.157 grams; or moist, 17.5 per cent; dried, 45.5 per cent.

From these the blood and spleen gave pure cultures of b. icteroides; the liver and pericardial fluid gave cultures contaminated with b. coli communis; the intestinal cultures were of colon and bac. proteus viridens.

Necropsy of mouse D.—Dead from b. icteroides from necropsy No. 18, New Orleans. Vessels of skin congested; inner surface of pelt and muscular and tendinous wall, of a good yellow tint; vessels of the mesentery engorged; liver very fatty, yellow with some hemostasis in dependent portion; left heart contracted, right full of blood; fluid of pleuræ and pericardium yellow in tint; spleen a little enlarged; kidneys swollen; there is a pronounced yellow tint of the abdominal contents; urinary bladder empty; the stomach contains at least one spot of *free* hemorrhage, and the submucosa is stained; the duodenum contains two accumulations of free, "black vomit;" the lungs seem congested. Moist liver, 0.520 grams; dried liver, 0.123 grams; residue, 0.090 grams; fat, 0.033 grams; or moist, 6.35 per cent; dried, 27 per cent.

Cultures from organs and trachea and intestine. Of these the blood gave an unmixed tube of b. icteroides. The organism was also isolated from the *tracheal* mucus, but not from the intestinal mucus *free* from blood.

Necropsy of mouse E.—Dead from b. icteroides obtained from Private

Smith. There is yellowness of inner surface of skin; congestion of mesenteric vessels; liver a very bright yellow from fatty change; spleen not enlarged; kidneys swollen; heart in diastole, left ventricle empty; blood fluid; stomach contains black vomit, identical in appearance to that seen in man, the serum being absorbed by bibulous paper, and the changed blood remaining as dark fragments or granules in contrast; its mucosa is deeply blood stained, with punctate hemorrhages, and extravasations of large size; the duodenum is distended beautifully at several points with extravasated blood; urinary bladder empty; lungs seem normal, or of bright tint. Culture tubes planted from organs and fluids and the bloody intestinal content. Of these the heart and pericardial fluid gives pure culture of the b. icteroides; the intestinal tube plated gave the organism in pure colony. The liver examination gave for 0.894 grams moist, 0.305 dried; after extraction with ether 0.140 grams residue and fat 0.165, or 18.3 per cent of moist and more than 50 per cent of the dried liver. No necropsy could have been more typical than this one.

Necropsy of mouse F.—Dead from b. icteroides from Private Smith. There is yellowness of inner surface of pelt; fullness of skin vessels, also of the mesentery; the liver is yellow from fatty change; spleen is slightly enlarged; kidneys are swollen; the stomach contains black vomit, the same as in mouse E; the intestine contains in its upper folds numerous small collections of black blood; mucosa much stained and has upon it a grumous black deposit; there are numerous submucous extravasations; bladder is empty. Cultures from organs and fluids and intestine. From these the heart blood, spleen, and pericardial fluid gave a pure culture of b. icteroides; the tubes from intestinal bloody mucus gave the organism in mixed culture, but it was not plated out.

Mice G and H were fed, as stated above, on a pure culture of bac. "X" of Dr. Sternberg, obtained through the courtesy of Dr. Reed, of the United States Army laboratory, with the result that, while the others of this series, except coli communis, died in marked unanimity as to time, and exhibited the most marked similarity, except the one which died of typhoid infection, in all post-mortem appearances, they alone remained hearty and well and were returned to the cage in good order one month from the day of exposure, on which day all mice were treated *exactly alike*, i. e., 1 c. c. of a twenty-four-hour bouillon culture being placed in each cage, and none thereafter.

Necropsy mouse I.—Dead from b. typhosus (Krâl). Skin vessels congested slightly; inner surface of the pelt pale and clear bluish tint; vessels of the mesentery full; the liver is of a dark *slate* color; does not look fatty; spleen is enlarged and rather soft; kidneys are swollen from congestion; the bladder contains a little albuminous urine; the stomach and its mucosa are normal to the eye, the mucus white and glairy; the intestinal mucosa congested; heart and lungs normal to the eye. Osmic acid gives no liver degeneration. Cultures from organs, fluids, and intestines.

From these there were strange results; from the pericardial fluid a pure culture of the b. typhi abdominalis; from the blood (one tube), a pure culture (no other organism plated out) of bac. coli com., corresponding to that of Sternberg in that it coagulates milk on the third day; in lactose it forms gas, $\overset{2}{\mathrm{H}}\,\overset{1}{\mathrm{CO}_2}$, in one-sixth of the tube; in glucose, gas, $\overset{2}{\mathrm{H}}\,\overset{1}{\mathrm{CO}_2}$, in one-third of the tube; is only motile and not translatory; in Dunham's solution there is indol production; on potato the

growth is a brownish yellow expansion. The spleen in bouillon culture gives an actively translatory rod, afterwards plated out in purity as b. typhosus; from the mixed intestinal culture, plated, the organism was not obtained.

Mouse J was fed on b. typhosus (Krâl) together with mouse I; remained well for some time after the death of mouse I on April 1, and was inadvertently placed by an attendant in a *dryer jar* (No. 2 of this series), and was found dead in this "Smith jar" on the 17th of April, from which mice F and E had died, the jar remaining in series uncleaned.

Necropsy mouse J.—Must have died on the 16th, for there is decomposition which shrouds appearances; the liver is somewhat yellow and fatty; spleen of normal size; in stomach the mucosa is covered with bloody mucus; there are evidences of much congestion in duodenum. Cultures from blood and organs. Those from heart and pericardium are pretty pure of a translatory colon which soon lost its motility, and which fermented all the sugars. This was replated but the b. icteroides was not isolated.

Mice K^1 and L were placed in same cage and given bouillon culture of bac. coli communis, a culture obtained from the laboratory of the Marine-Hospital Service, and which corresponded in all respects to the bac. "X" of Sternberg and to that of Havelberg, on the 23d of March, 1899. On the 25th L was missing from the cage and never seen again. However, from the plethoric condition of the mouse K^1, it is thought that she ate the smaller L.

Mouse K^1 was alive and perfectly well on the 24th of April, or one month from date of exposure, and remained permanently so.

This series of necropsies proves the acute infectious character of bac. icteroides to rodents; that the post-mortem appearances are the same in all cases; that they resemble closely those observed in the human body after death from yellow fever; that bacillus typhosus is less markedly infective, and the post-mortem lesions are less marked; that with bac. icteroides the "secondary infection" by other germs is not so marked, the blood and organs giving, in many cases, pure culture of the organism, a few giving also colon, or proteus; that staphyloeocci and streptococci were practically unknown in these cases; that in all except mouse I the hepatic cells were extensively fatty, teased fresh preparations in osmic acid showing this change, while in that of I there was only cloudy swelling, with further necrosis; finally, that while bac. "X," and others of the common colon group, when injected into the tissues of these little animals do give rise to a more or less fulminant illness and death, the organs showing a marked degree of steatotic change, the stomach containing at times "black vomit," there is no infectiveness attaching to them either for man or animals—i. e., they are pathogenic under artificial conditions, but not naturally, or infective.

Necropsy mouse K^2.—Dead from b. icteroides from Smith, April 27, noon. Much congestion of the vessels of the skin; yellow tint on its inner surface; tissues are dry; vessels of mesentery engorged; liver is very yellow from fatty change; spleen is normal in size, friable, and dry; lungs are much congested, and there is some lobular pneumonia; right heart full of liquid blood; kidneys swollen; stomach contains only a little "black vomit," no débris of food; duodenum contains free blood; urine is albuminous; liver, moist, 1.220 grams; liver, dried, 0.318 grams; residue, 0.188 grams; fat, 0.130 grams; or moist 10.6 per cent, dried 40.9 per cent. Cultures from organs and fluids; from which heart and spleen give pure cultures, b. icteroides; the others are mixed from trachea, liver, and pericardial fluids.

Necropsy mouse K[3].—Dead from b. icteroides from Smith, April 20, 1899. Vessels of skin and mesentery intensely engorged; liver very fatty; spleen normal in size; stomach and duodenum contain free blood as "black vomit;" the lungs are congested; cultures from blood from which the organism was plated in pure culture.

Necropsy mouse L[1].—Dead from b. icteroides from Smith. Tissues dry; vessels engorged; those of the mesentery, also; liver very yellow and fatty in appearance; spleen normal in size; heart in diastole; lungs congested with patches of broncho-pneumonia; kidneys are congested; the bladder entirely empty; the stomach mucosa has many punctate and free hemorrhages; small intestine the same; cultures from organs and fluids.

After forty-eight hours the spleen and liver give pure cultures of b. icteroides; the blood of heart gave the organism mixed with a straight motile (translatory) bacillus twice as long as that of Sanarelli. There were some colonies from the trachea and bronchi, but these animals are so small that it is impracticable to enter these tubes without the danger of encountering free blood, which, containing the bacillus, questions the integrity of the observation that the organism exists in the tracheal or bronchial mucus in pure culture.

Mouse M[1] died from b. icteroides and fed to domestic pigs, without necropsy.

Necropsy mouse M[2].—Dead from b. icteroides, from Smith through M[1]. Dead five minutes; tissues dry and yellowish; mesentery engorged; liver yellow; spleen slightly swollen; kidneys swollen; lungs congested; left heart firmly contracted; right full of blood; ecchymoses on diaphragmatic pleura; bladder empty; the stomach contains 2 c. c. of a dark-brownish fluid, with flakes of black substance (black vomit); the mucosa extravasated at many points; small intestine has some free blood. Liver, fresh, 1.100 grams; liver, dried, 0.350 grams; residue, 0.165 grams; fat, 0.185 grams; or moist, 17 per cent; dried, 53 per cent. Cultures from all organs and fluids.

The organism of Sanarelli is regained from blood and spleen. From the trachea and tubes it is in mixed cultures.

Necropsy mouse N.—Dead from b. icteroides. Liver is very yellow and fatty to cut and feel; stomach contains blackish fluid; mucosa is extravasated; urine albuminous; cultures from blood and spleen, from which results a pure culture from spleen; organs fed to two domestic pigs.

Necropsy of brown rat fed on mixed culture from stomach of Williams, dead from yellow fever.

Vessels of skin full; tissues dry and yellowish; inguinal lymph glands enlarged; vessels of mesentery engorged; liver is congested and presents plaques of bright yellow change, with red areas; there is a large tapeworm encysted between the lobes; the stomach mucosa shows numerous punctate hemorrhages, some of them free on the surface; duodenum the same; spleen normal in size; kidneys congested; urine albuminous; lungs studded with cheesy deposits, some of them as large as buckshot; many of these upon visceral pleura; the glands at the roots of the lungs generally enlarged; there are also numerous cysts containing parasites; heart full of fluid blood; cultures from organs and fluids; cover slips from cheesy material. The jaws were broken and mucus from trachea and bronchi obtained *free* from blood.

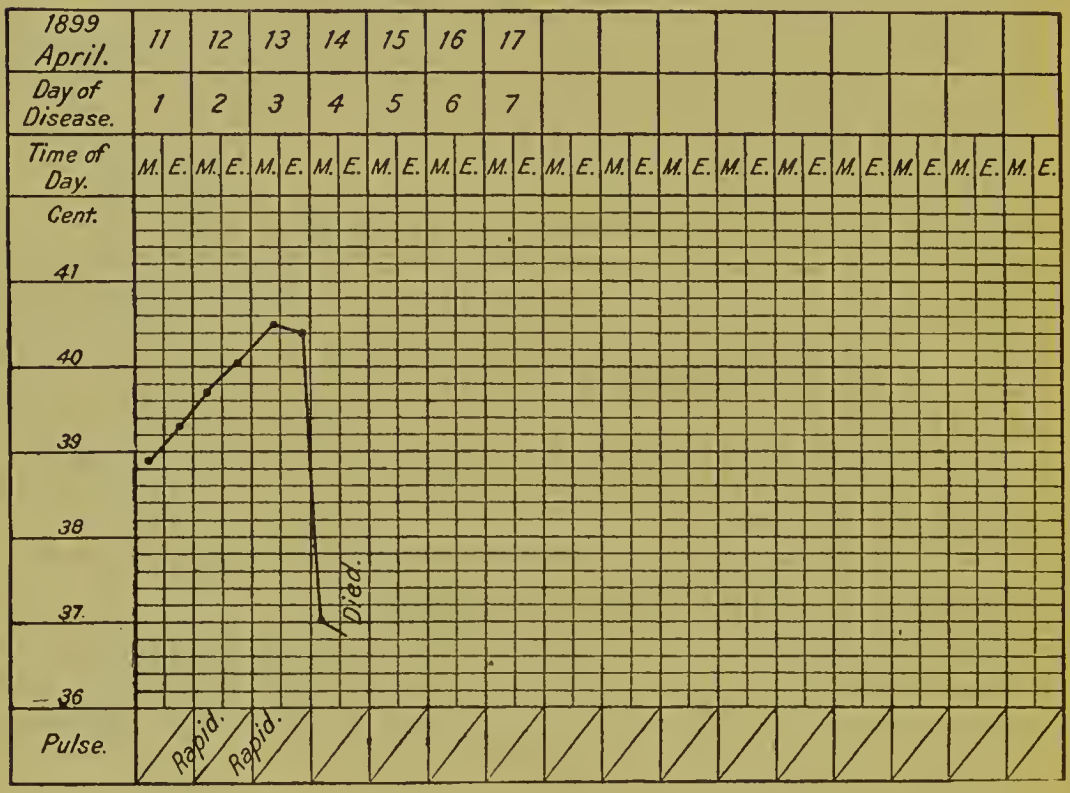
From these cultures the spleen gave a pure colon, at first translatory, but later permanently immotile; other tubes sterile. From the bronchial mucus we isolated readily the b. icteroides in pure culture. The covers stained showed no tubercle bacilli in cheesy nodules. This was

the only b. icteroides that we were able to isolate from this case, and the possibility of its having gained entrance to this cage from some others near it must be considered, and especially would the disease of the lungs predispose it to infection.

OTHER EXPERIMENTS CHIEFLY BY INSUFFLATION.

In the endeavor to submit animals to the influence of the bac. icteroides in as natural a manner as possible the twenty-four hour old culture, scraped from large agar plates, was mixed with lycopodium powder in good proportion and slowly dried at incubator temperature. This could be used at the end of twenty-four hours in an ordinary powder blower, the animals being confined in a close box, the tube nozzle passed through a small opening. Insufflations were generally of three-minute duration.

[Dog A.—Disease, yellow fever.]



On March 10, 1899, a good-sized dog, A, was fed with food impregnated daily with b. icteroides from several sources until the 25th, when she appeared quite well. On the 25th we incised the abdominal wall (laparotomy) and, withdrawing a loop of the small intestine, carefully injected into its lumen 2 c. c. of a twenty-four hour bouillon culture of b. icteroides, from No. 18 New Orleans, under chloroform. Reaction was good. On 26th no bad results, eats well; 27th no bad results, eats well; 28th no bad results, eats well. April 1 she is well. On April 4 she was chloroformed and tracheotomized and a tube placed in trachea. On the 7th she is quite bright and eats well, and tube in good condition. At 1 p. m. the lungs were insufflated with a freshly prepared powder, bearing the b. icteroides (from Smith). Until the morning of the 11th she gave no sign of illness, but then we were informed that she was sick, and found that she had aborted a nearly full-grown pup, and was quite ill, there being free hemorrhage. Temperature high; pulse rapid. On the 12th she is very ill; lies down,

and does not notice food; there is a free hemorrhage. Blood taken from ear vein and planted. On 13th blood again planted. On 14th dead at 9.30 a. m.

Necropsy (at once).—Tracheotomy tube clear and no hemorrhage or pus; old abdominal incision nicely healed; tissues dry; mesentery very fatty, obscures congestion; the liver presented a perfect appearance of yellow, fatty change, with dilated venous radicles imparting a "nutmeg" appearance; there were large, bright yellow plaques, and it was generally yellow when cut; gall bladder was full of clean bile; spleen normal in size and dry; kidneys swollen and on incision gave perfect pictures of fatty change in the cortex at the bases of the pyramids; bladder was almost empty, the few drops of urine becoming solid albuminous flakes when dropped into picric acid solution (Esbach's); there was some free blood in the abdomen from a rupture of a small vessel in the gastro-duodenal mesentery; pericardium was ecchymotic on both layers, some spots being 1 cm. in diameter; they were most numerous upon the visceral layer; the fluid was increased and yellow; the heart muscle was pale, left ventricle contracted, right full of black fluid blood; the diaphragmatic pleuræ were ecchymotic, also the visceral layer; fluid scant and yellow; the lungs were contracted and of a deep red color; the bronchial mucosa inflamed and bathed in muco-pus; the stomach contained 250 c. c. of a "dark grumous fluid," or black vomit; the mucosa was ecchymotic and stained, covered with a glairy mucus which entangled numerous flakes of the blackened blood; the duodenum gave the same picture, the small intestine beyond being intensely engorged, with here and there free blood; the uterus was enlarged; its peritoneal covering ecchymotic; one horn was full of clot and placenta, the other was hemorrhagic and empty. Moist liver, 10.000 grams; dry liver, 2.676 grams; residue, 1.658 grams; fat, 1.018 grams, or 10.18 per cent wet, 38 per cent dry. Cultures from all organs, blood, bile, and urine and fæces. The cultures from living blood rested sterile. The liver and urine gave pure cultures of other organisms; the other tubes were sterile. That of the liver was a very coarse rod, motile, truncated, and growing on agar as a dense opaque layer, assumed to be a hay bacillus of accidental contamination; that from the urine was an immotile bacillus, having no similarity to b. icteroides. Growths from the fæces were plated with no result, so that b. icteroides was *not* isolated from this animal; the bronchial mucus was not planted. From the time elapsed between the feeding experiment and the introduction of the germ *beyond* the acid stomach into the intestine, as well as the subsequent typical period of incubation, from the 7th to 11th, and inception of the disease, its typical course, and ending with so perfect a necropsy, it must be assumed that this was a pure, or natural, infection, the insufflation having been an exaggerated *inspiration* of the bacillus. The absence of all organisms from the blood and organs (the mucus from the trachea and tubes was unfortunately overlooked) was readily attributed to the well-known bactericidal power of the serum of these animals; but later observation showed that this was also the case in rabbits, whose serum is not so potent. Professor Sanarelli always exhibited his organism in dogs intravenously, and, of course, in great numbers, since as high as 20 c. c. were at times injected, most of his experimental records showing that death was due to the toxins injected, an intoxication, rather than to the bacillus, and that their presence in the blood and tissues was simply because they were in such numbers as to resist destruction, a number of his dogs requiring *several* intravenous injections. This would not have been the case if a true multiplication of the bacillus had taken place, an active sep-

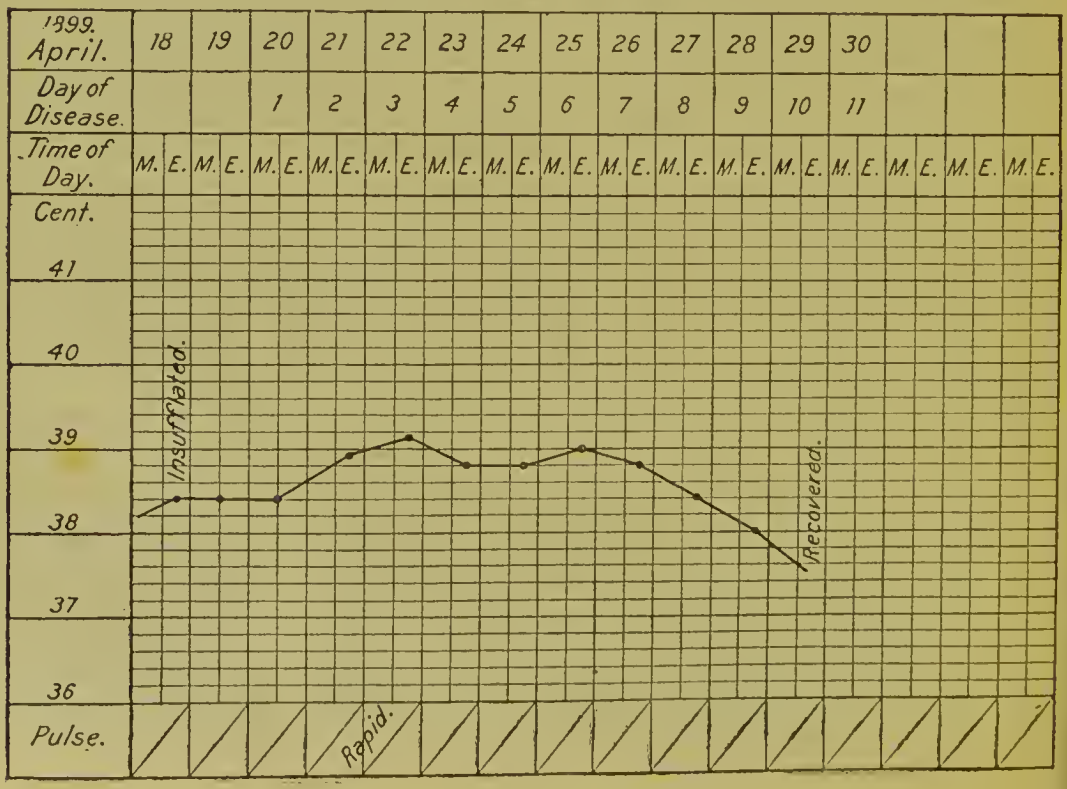
ticæmia, and it is probable that this can not take place in the dog, or, in other words, that the dog, naturally very resistant to yellow fever, does not develop naturally a septicæmia as a "secondary infection" in this disease. In dog A the disease followed a natural, if slightly exaggerated, process, and from 5 p. m. of the 13th, when she was so nearly dead that we feared a demise during the early hours of the night with the "secondary invasion" from *possibly* the alimentary canal of a horde of organisms, until her death, at 9.30 a. m. of the 14th, there was surely ample time in these "last hours of life" for this secondary invasion. She was operated upon while still warm; a few hours post mortem this invasion might have occurred. Had Sanarelli used cultures of the bac. coli communis the same results would have been reached as with his experimental methods in dogs, and the same can be said of his experiments with the filtered cultures (toxins) in man, that while they proved *toxicity*, there was absolute lack of proof that b. icteroides was *infectious*. Since two different bacilli can give rise to similar intoxications, the one can not be called more or less than the other the specific cause of an acute infectious disease unless the experiment shows the manner of such infection to be one not natural to the other.

On April 7 we placed a young guinea pig of 250 grams and a rabbit of 700 grams in a close box with a small opening for insufflator tube, and insufflated them for ten minutes. When removed from the box their coats were well dusted with the powder. On the 8th this was repeated for five minutes. On the 17th they were perfectly well and returned to general cage.

On same date we tracheotomized a young rabbit and introduced a glass tube, under ether. On the 8th insufflated the lungs through the tube for several minutes with the powder containing the bacillus icteroides. On the 9th the animal died suddenly from choked tube. All organs and blood sterile. (Lung not examined.)

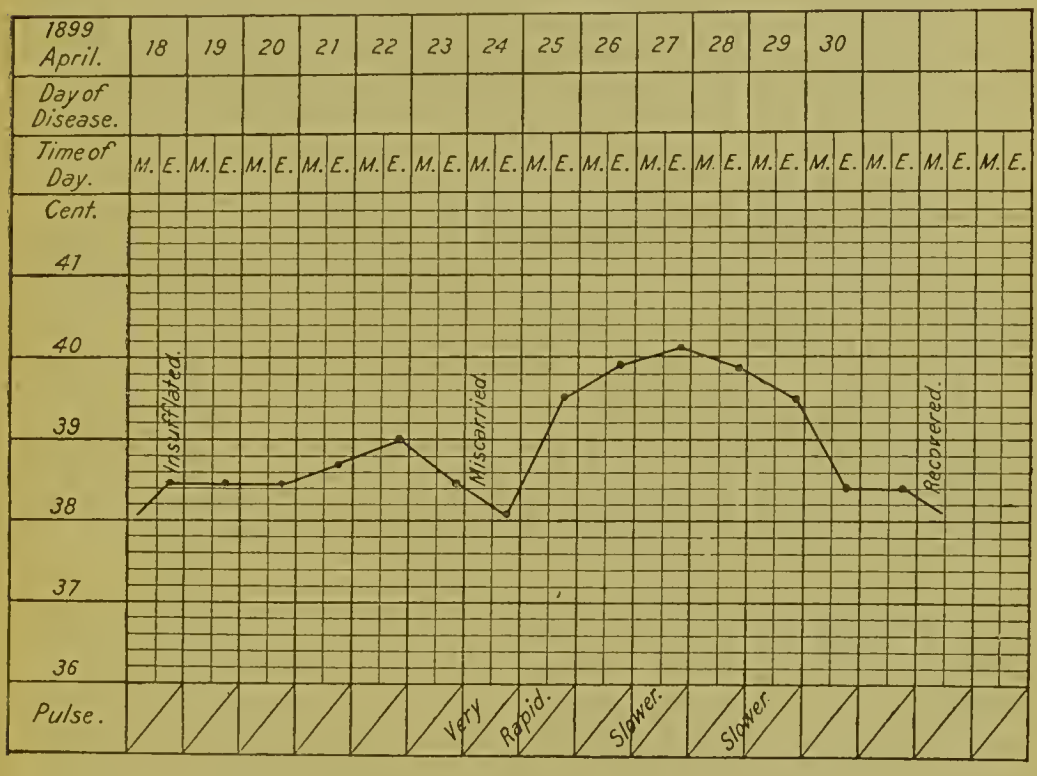
On April 15 and 16 we tracheotomized as above three dogs under chloroform; all reacted well.

[Dog B.—Disease, yellow fever.]



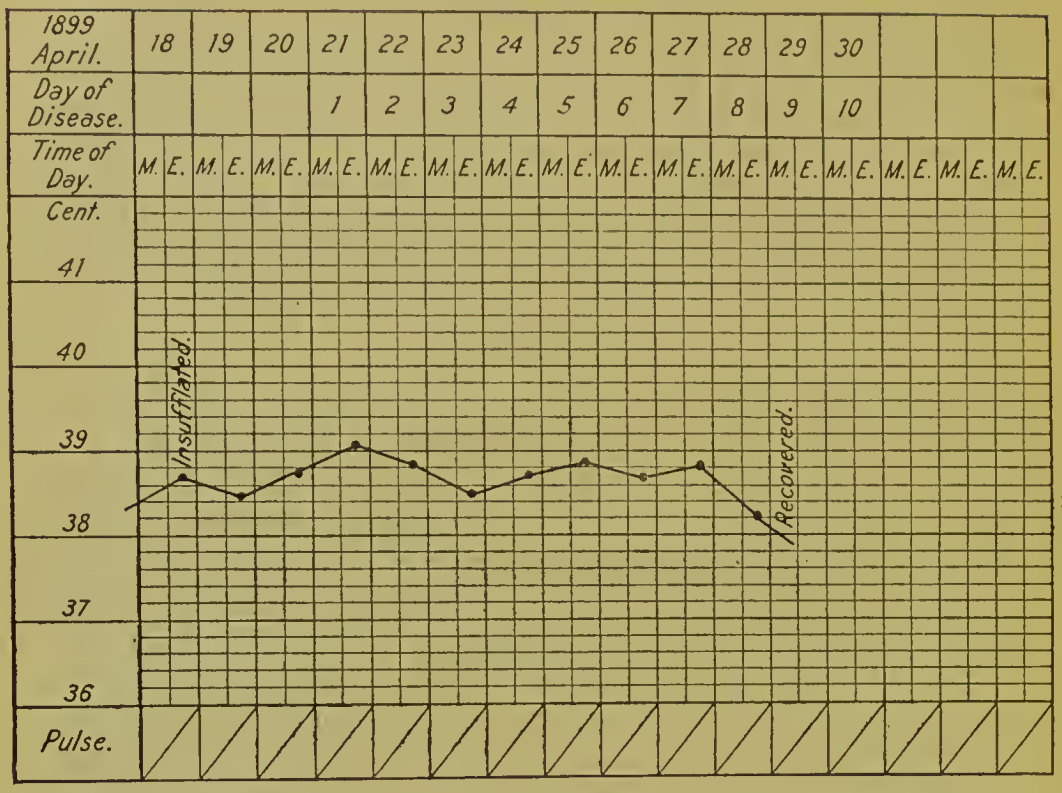
April 18, dog B, a plump male of 7 kilos, native; wound partially healed and in good condition; was insufflated with b. icteroides in lycopodium for two minutes; vomited in ten minutes. April 19, doing nicely; again insufflated two minutes. April 20, doing nicely; eats heartily. April 21, doing nicely; eats fairly. April 22, doing poorly; eats little; seems sick; nausea. April 23, doing poorly; eats little; is right sick. April 24, doing poorly; eats little; blood taken from ear. April 25, doing poorly; eats little; reclines a great deal. April 26, doing poorly; eats little; more active. April 28, doing well; eats much better; recovered; blood from ear rested sterile in bouillon.

[Dog C.—Disease, yellow fever.



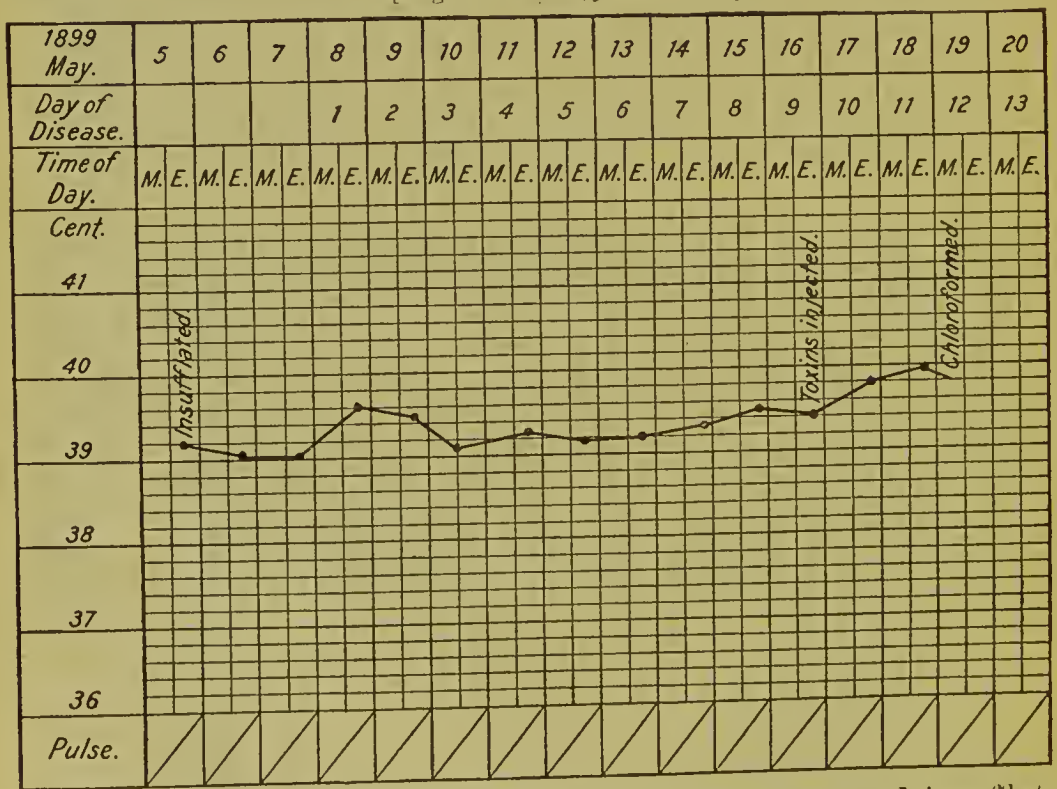
April 18, dog C, a pregnant female of 5,850 grams, native; was insufflated through tube with bac. icteroides in lycopodium for two minutes. April 19, fairly well; tube clear; again insufflatedtwo minutes. April 20, fairly well; eats heartily. April 21, fairly well; eats heartily. April 22, fairly well; eats little; blood taken. April 23, fairly well; eats little. April 24, only poorly; pupped five pups; look premature. April 25, 26, and 27, only very poorly; pups not cared for; two dead; blood taken. April 28, only poorly; does not eat; blood taken. April 29, only poorly; eats a little and cares for pups. April 30, better; eats well and cares for pnps. May 8, she is in fine condition; another pup accidentally killed; ear-tip blood always sterile in bouillon.

[Dog D.—Disease, yellow fever.]



April 18, dog D, small tan female, 4,370 grams; insufflated with b. icteroides for three minutes; April 19, has lost her tube; April 20, eats well; April 21, eats well, seems quiet; April 22, eats poorly; April 23, eats poorly; April 24, eats poorly; April 28, eats well, seems as usual. Ear-tip blood always rested sterile in bouillon.

[Dog G.—Disease, yellow fever.]



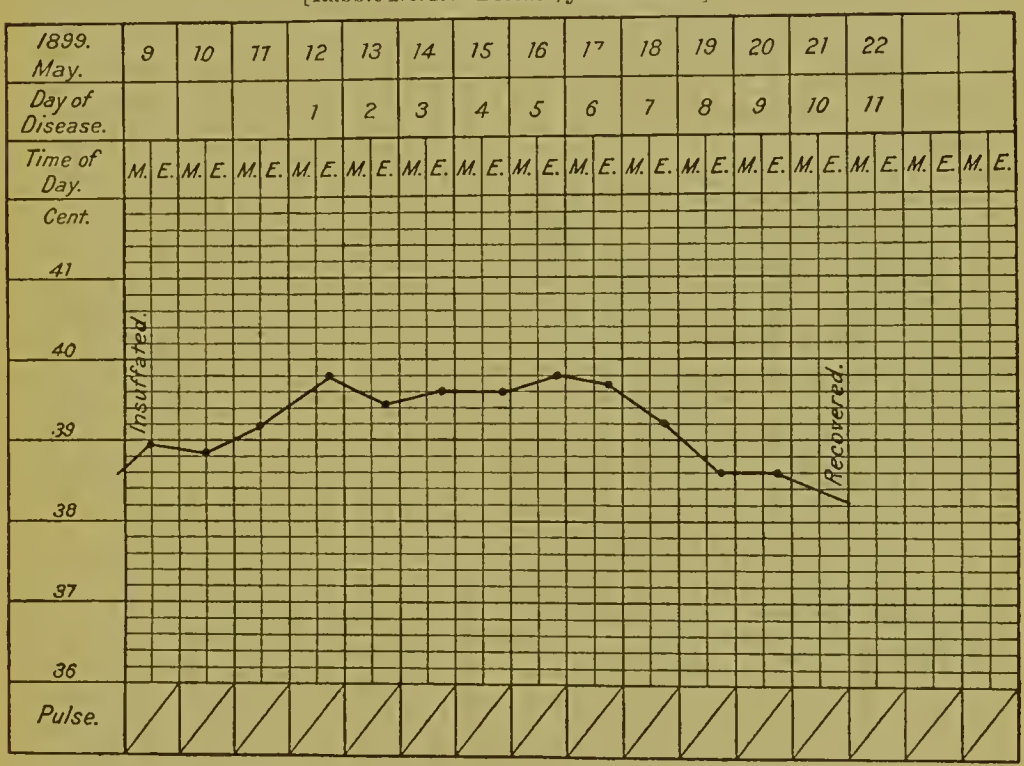
May 5, dog G (a Tampa reagent), was chloroformed and insufflated through the larynx with b. icteroides for two minutes. May 6, eats very

little and appears sick; May 8, eats better, appears better; May 9, eats but little; May 11, eats but little, coughs much; May 12, eats little, is ill; May 15, eats little, coughs much; May 16, eats a little better; gave 5 c. c. soluble toxins and there was immediate nausea and other symptoms; May 17, eats a little, is better, coughs much; May 18, eats nothing, is quite sick. He was chloroformed.

Necropsy showed normally colored liver and enlarged spleen; mucosa of stomach injected; from the duodenum to the ileo-cæcal valve the intestinal mucosa was much congested, and here and there were minute extravasations; visceral pleuræ ecchymotic; lungs deeply congested; mucosa of tubes normal, some deep mucus; bladder has 25 c. c. urine, slightly albuminous. Moist liver, 7.465 grams; dry liver, 1.910 grams; residue, 0.370 gram; fat, 0.540 gram, or 7.2 per cent of the moist and 28.3 per cent of the dried liver. Cultures from blood and organs all remained sterile, even the *bronchial mucus*.

In this case death would probably have ensued from the exhibition of the soluble toxins, since to it alone can be attributed the anatomic results.

[Rabbit No. 29.—Disease, yellow fever.]

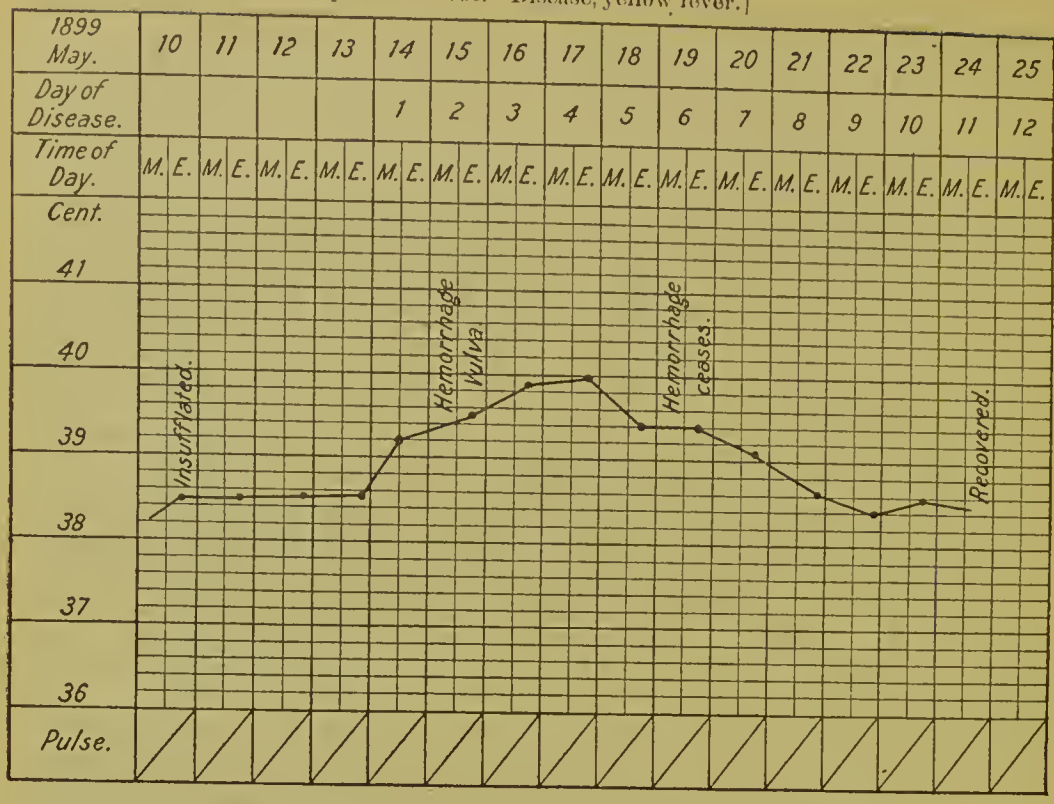


May 9, insufflated through larynx, without anæsthetic, with b. icteroides, a large buck rabbit, No. 29. May 10, appears well; May 11, appears droopy, eats little; May 12, appears droopy, eats little or none; May 13, appears very droopy, dyspnœa, eats little or none; May 14, appears easier; May 15, appears easier, eats little; May 17, appears easier, eats better; May 20, appears well.

May 10, No. 30, a medium-sized female rabbit was insufflated without anæsthetic, with b. icteroides for three minutes. May 13, very quiet, eats little; May 14, very quiet, eats little; May 15, very quiet, lying down, free hemorrhage from vulva; May 16, very poorly, lying down, eats none, bleeding; May 17, very poorly, lying down, eats little, bleeding; May 18, very poorly, lying down, eats little; May 19, very poorly, lying down, eats little, no more blood; May 22, much better, eats some; May 25, seems well, eats well.

These two animals reacted very slightly; they were sick, as the charts

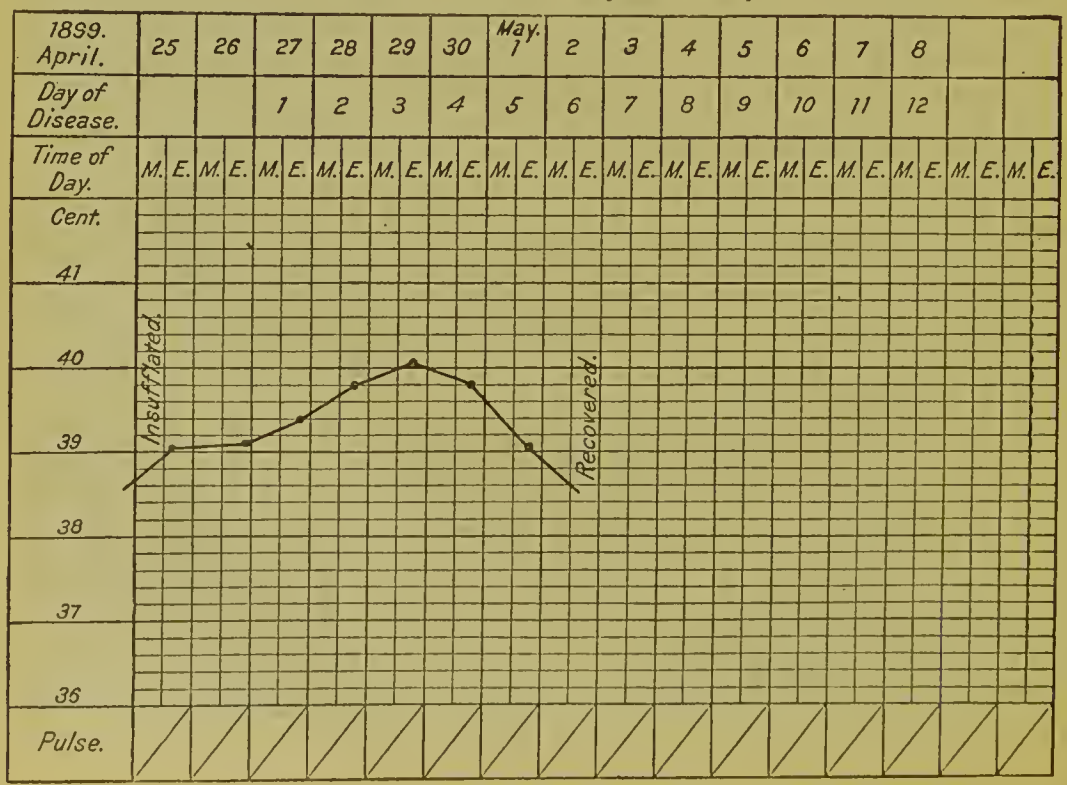
[Rabbit No. 30.—Disease, yellow fever.]



show, but it was very mild. Blood taken on the 15th and 16th and planted in bouillon gave no growths; that is, there was no septicæmia.

May 9, dog H, a small female, of 3 kilos, native of Florida, was insufflated through the larynx without anæsthetic. From this date to 17th the temperature ranges from 38.6° to 39°. There is *no* reaction; the dog eats well and is well. No result. May 20, injected into trachea of same dog through the skin 1 c. c. suspension b. icteroides. There is no reaction in temperature. There is a paresis of right fore leg and a chronic spasm of muscles of right shoulder, yet she walks, but reclines much. May 27, eats well; temperature, 38.7°; she is running any where; well.

[Rabbit No. 40.—Disease, yellow fever.]

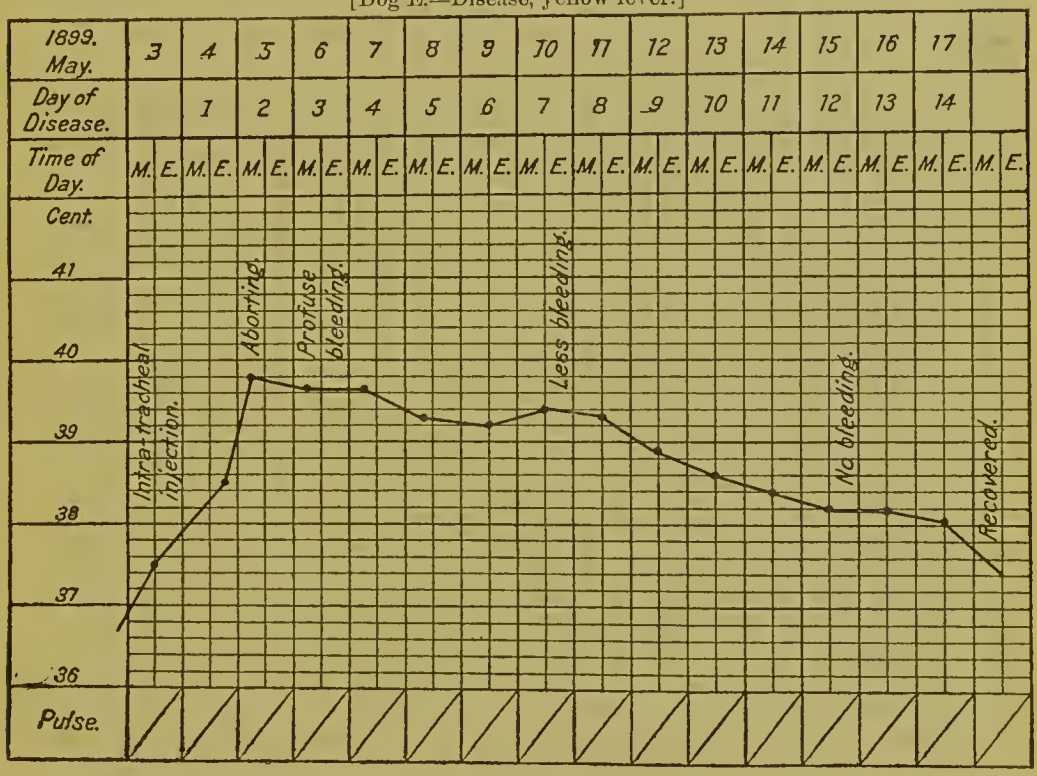


April 25, insufflated the trachea, through larynx, under ether, of two rabbits, one a large male from Washington, the other a small female, No. 40. During the fever this animal ate very little and became emaciated. Blood drawn proves sterile. May 1, recovered. The large male scarcely reacted, his best temperature being 39.8°; his normal about 39.3°.

We believe the fumes of the ether influenced these cases and discontinued its use; also, that the condition of the organism, whether in dust or fluid, matters little, so long as the bacillus reaches the tubes. We gave some intratracheal injections of b. icteroides in bouillon.

On May 3, 1899, dog E, large female, pregnant; weight, 23 kilos.; a native of Florida and nonimmune; temperature, 37.5°; was etherized and the trachea exposed, into which, by means of a sterile syringe, 0.5 c. c. of bouillon culture of bac. icteroides was injected, the needle wound being treated with biehloride as the instrument was withdrawn. May 4, appears well, eats heartily; 5 p. m. she has chills. May 5, at 8

[Dog E.—Disease, yellow fever.]



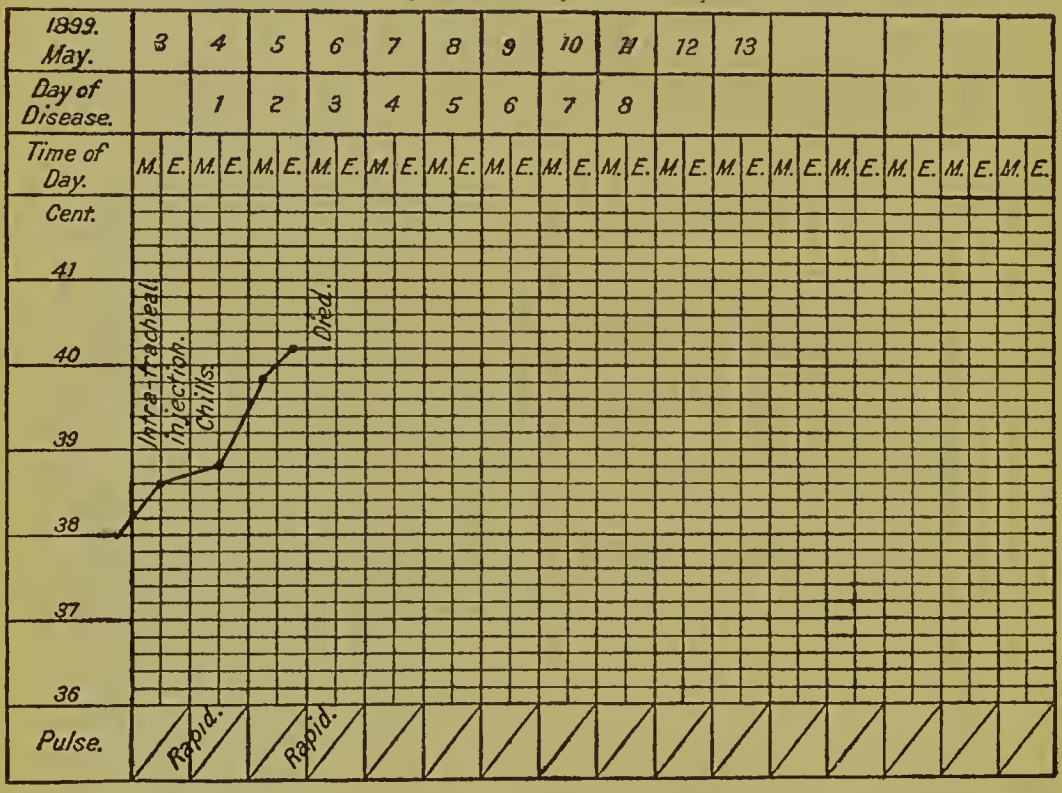
a. m. she is aborting and eats the embryos. May 6, at 10 a. m she is ill and flooding profusely and lies down continually. May 7, at 10 a. m. she is up and eats a little; some blood. May 10, at 10 a. m. she is better; some blood. May 15, at 10 a. m. she is better; no blood. May 17, practically well, and eats well; no blood.

In view of the experience with dog A, into whose jejunum was put 2 c. c. of bouillon culture of b. icteroides without any symptoms whatever, we must conclude that the symptoms in this animal were not altogether due to the small amount of toxin in the 0.5 c. c. injection, but to an increase of the organism introduced and the quick response to the rapid increase of an already formed (in bouillon) colony.

Dog F, the same as dog B, still wearing his tracheal tube, was injected through the tube with 0.5 c. c. bouillon culture b. icteroides on May 3, 1899. May 4, animal seems as usual; eats very little. May 5, animal at 8 a. m. has chills; nausea, tracheal secretion. May 5, at 2.30 p. m. died in convulsion.

Necropsy (*half hour post-mortem*).—There is rigidity; lividity of lips and tongue; skin vessels full; those of mesentery engorged; liver deeply congested, but yellow in large plaques; spleen is enlarged; kidneys are congested; bladder is empty; the pleurae have ecchymotic spots; the lungs are much affected; of the right lung the two lower lobes were deeply congested and there was broncho-pneumonia, the lung floating very deeply in water bath; the left lung gave a semisolid lower lobe; the trachea and bronchi were full of a mueo-pus; the mucosæ ecchymotic even into the smaller tubes; pericardium and fluid normal; right heart full of black fluid blood; the stomach contains 100 c. c. of dark bloody fluid; the mueosa is extensively extravasated; in the duodenum the picture is the same. Cultures from everything. In forty-eight hours' incubation this dog's organs and blood prove *sterile*, as in A. The eultures from trachea and tubes and cut lung

[Dog F.—Disease, yellow fever.]



surface gave the b. ieteroides injeeted, mixed with other organisms. Liver teased in osmie acid showed much fat. Fat in fresh liver, 9 per cent; dried liver, 33 per eent.

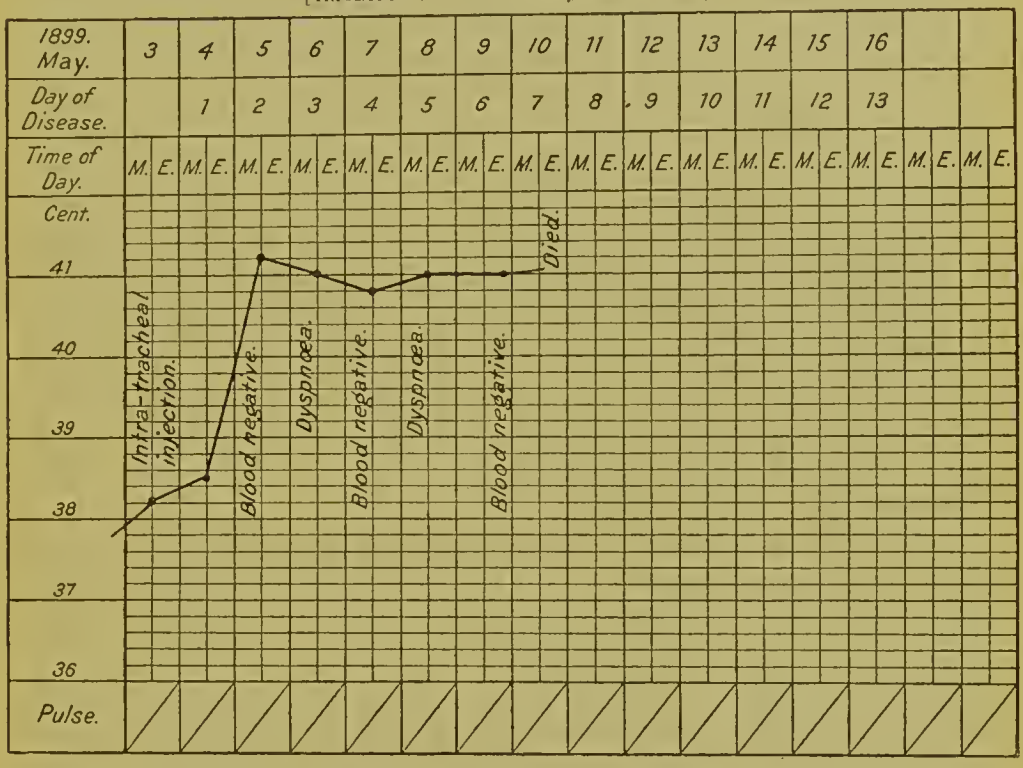
The prompt reaetion in this ease, I think, arose from the fact that this dog had had some difficulty in wearing his tube, and the lungs were in a condition of semicongestion most suitable for infection. We can not explain the questionable reaction in the case of this dog as B.

On May 3, 1899, a small rabbit, No. 15, was etherized, the trachea exposed by incision, and 0.5 e. e. bouillon-culture b. ieteroides (Smith) injected by syringe into its lumen; the wound carefully disinfected; reaction, good; temperature, 38.3°. May 4, animal eats well, seems well. May 5, animal eats little, is sick; dyspnœa. May 7, animal eats nothing; breathing badly. May 8, animal eats nothing; blood planted. May 9, animal is dead at 8 a. m.

Necropsy (*at once*).—Much emaciation; rigidity; vessels of mesentery engorged; liver deeply eongested, but *not* yellow; spleen, normal; kidneys, congested; right heart, distended; the pericardium purulent; pleural fluid opalescent, membranes inflamed; the lungs deeply con-

gested, with scattered points of a lobular pneumonia; tubes contain a free muco-purulent secretion; the stomach contains a bolus of undigested food, deeply stained from numerous free hemorrhages from the mucosa; that of duodenum deeply congested and extravasated; urinary bladder is empty; cultures from all sources. This is not a characteristic post-mortem, still the hepatic cells teased in osmic-acid solution showed numerous black granules of fat in a larger number of them; others were granular, with fragmenting nuclei. After forty-eight hours' incubation, the blood drawn and planted on the 8th remained sterile. The tubes from pericardial pus and pleural fluid gave pure cultures of b. icteroides, the pus carried into various media giving all demands of

[Rabbit No. 15.—Disease, yellow fever.]



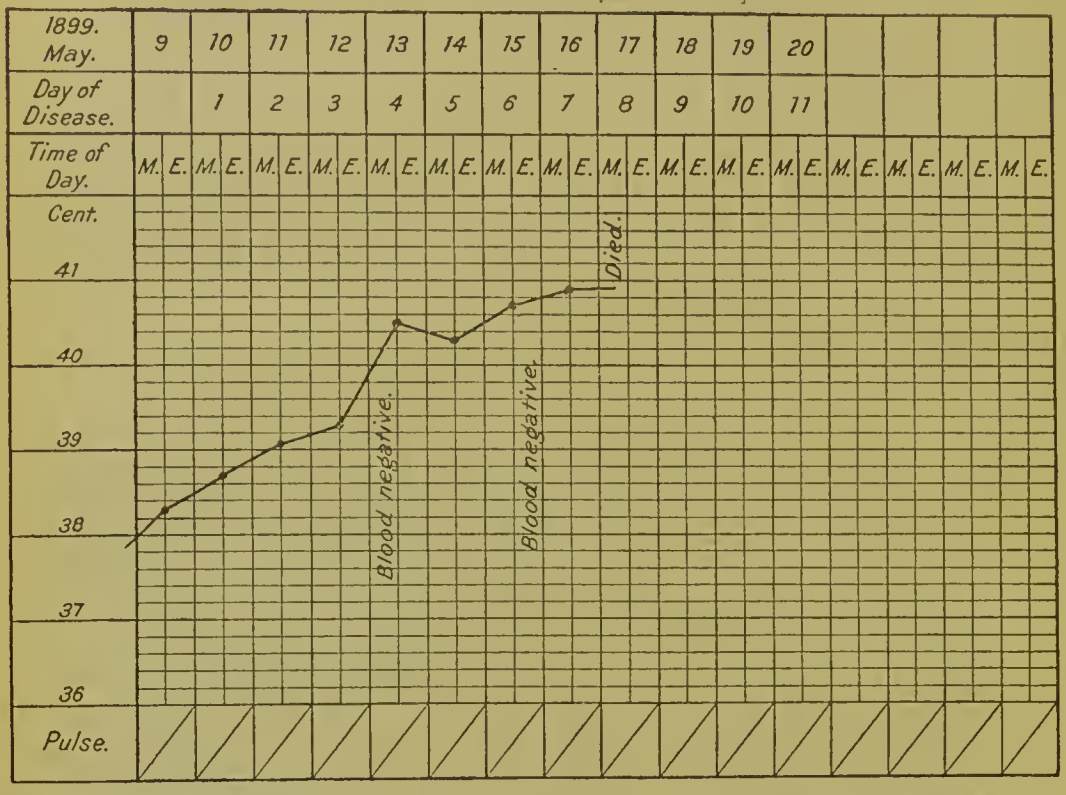
Sanarelli. *All other tubes sterile.* Stained cover slips of this pus gave numerous rods among the leucocytes, many of them already engulfed by the large multinuclear cells. There was *absolutely* no other organism in these fluids.

May 9, exposed trachea of a small rabbit (No. 25) and injected into its lumen 0.5 c. c. of bouillon culture of b. icteroides (Smith). May 10, animal coughs and eats little. May 11, animal crouches; eats little, and coughs. May 14, animal lies all the time; eats nothing; coughs. May 15, animal lies all the time; eats little; blood planted. May 16, dies at 4 p. m.

Necropsy (at once).—Much emaciated; vessels of skin and mesentery engorged; liver somewhat yellow; spleen normal in size; pericardium normal; right heart distended; left contracted; the trachea and bronchi inflamed and full of muco-pus; pleuræ ecchymotic; the lungs show many lobules shrunken from pneumonic change; the mucosa of the stomach is ecchymotic, as is that of the duodenum; there is no free hemorrhage; urinary bladder empty; gall bladder full; cultures from all organs and fluids. Moist liver, 8.660 grams; dried liver, 1.689 grams; residue, 1.305 grams; fat, 0.384 grams, or 3.8 per cent wet and 22.8 per cent dry.

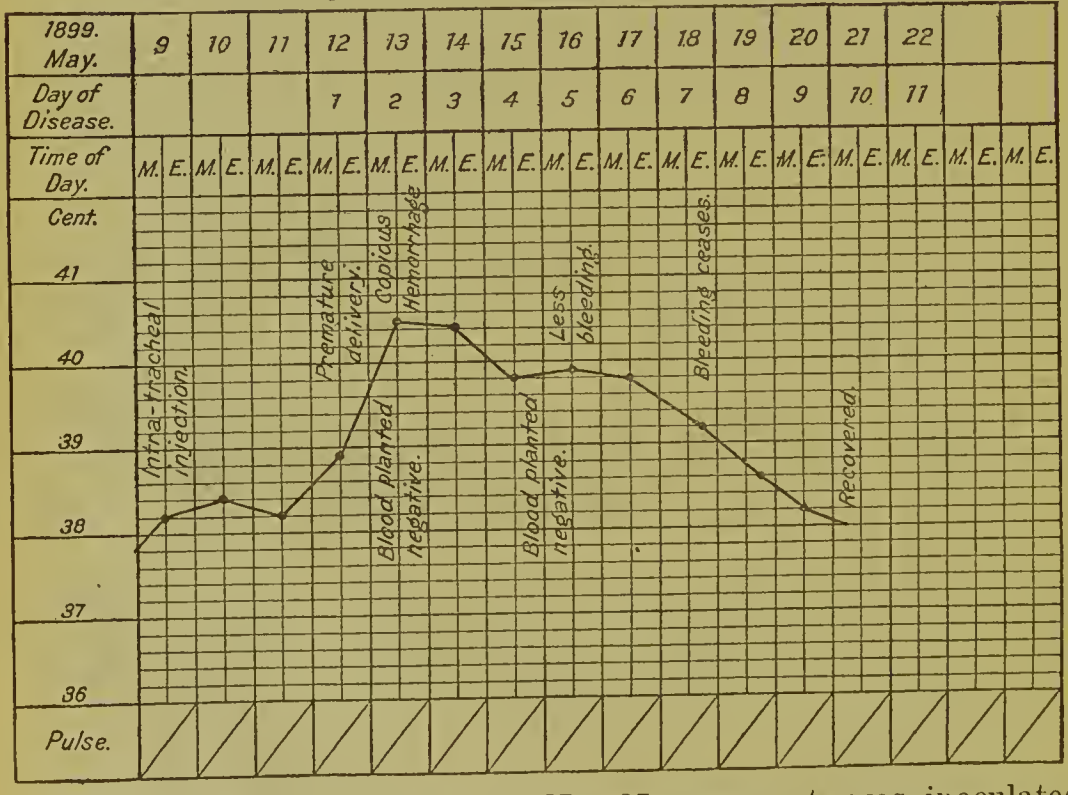
After forty-eight hours incubation *all tubes remained sterile* except those taken from the *trachea*, *tubes*, and *lung surface*, which gave the b.

[Rabbit No. 25.—Disease, yellow fever.]



icteroides in mixed culture, from which it was readily isolated pure. Blood planted on 13th and 15th *remained sterile.*

[Rabbit No. 27.—Disease, yellow fever.]

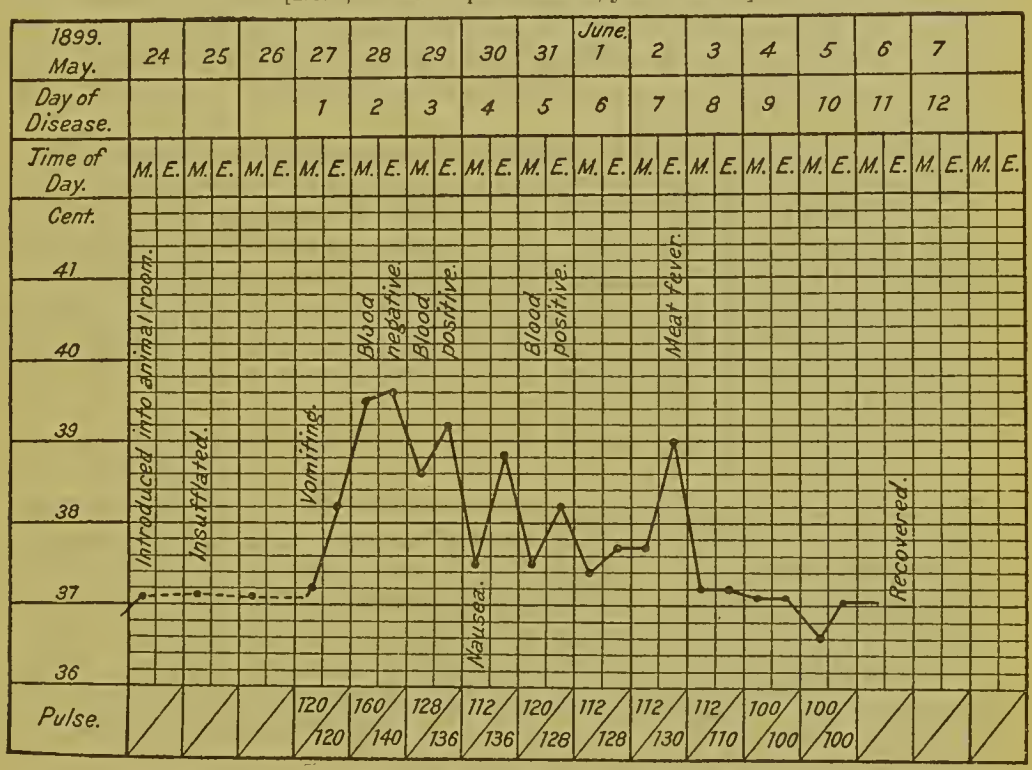


May 9, a large female rabbit, No. 27, pregnant; was inoculated through the trachea with 0.5 c. c. bouillon culture b. icteroides. May

10, animal quiet. May 11, animal quiet; eats some. May 12, animal gives birth to eight young; immature. May 13, animal has bloody lochia; eats little; blood planted. May 14, animal has bloody lochia; eats better. May 15, animal seems better; eats better; blood planted. May 17, animal seems better; cares for young nicely. May 20, animal is well; two pups are dead from starvation; the others are very emaciated; four are placed on the bottle; all died.

May 23, 1899, at 6 p. m., introduced into our animal room a large black ape for experimentation. May 25, at 10 a. m., the trachea was insufflated through the larynx for three minutes with lycopodium powder bearing b. icteroides in good quantity. There was no doubt of its having been inhaled, since the animal sneezed and coughed freely. Temperature, 37.3°. May 26, again insufflated freely. During afternoon the animal seems dull. May 27, at 8.30 a. m., it has a vomiting spell; appears dull; does not eat much. Axillary temperature, 37.4°;

[No. 1, African Ape.—Disease, yellow fever.]



pulse, 120. Again insufflated for several minutes. May 27, at 5 p. m., axillary temperature, 38.4°; pulse, 120; slight diarrhea; is restless and somnolent. Milk diet. May 28, 9 a. m., temperature, 39.5°; pulse, 160; is quite ill; lies down and takes little notice. Blood from ear tip planted. Does not touch food. Five p. m., axillary temperature, 39.6°; pulse, 140; seems very weak and depressed; has craved water all day. May 29, 9 a. m., temperature, 38.6°; pulse, 128; animal is not better this morning; seems quite ill; much thirst; lies down. Blood from ear tip planted. Five p. m., temperature, 39.2°; pulse, 130; some diarrhea.

May 30, 9 a. m., temperature, 37.5°; pulse, 112; eats a lump of sugar and chews sugar cane, but will not touch food. At this hour he is brighter, swings on his ropes, and is becoming interested. Milk punch. At 2 p. m., he is again suffering from nausea, curls up on his bed, and is indifferent to all comers alike. At this hour, temperature, 38°; craves

water; blood planted. 5 p. m., temperature, 38.9°; pulse, 136; about the same. 10 p. m., temperature 38.6°; pulse, 136; about the same.

May 31, 9 a. m., temperature, 37.5°; pulse, 120; seems better; eats nothing. 5 p. m., temperature, 38.2°; pulse, 128; notices occurrences in the room, but does not eat; is much emaciated and feeble. In fact, the results of his sickness are striking. Blood again planted.

June 1, 9 a. m., temperature, 37.4°; pulse, 112; is much brighter, but very weak; does not care for food. 5 p. m., temperature, 37.7°; pulse, 128.

June 2, 9 a. m., temperature, 37.7°; pulse, 112; he is better and looks better, but has no appetite; drinks coffee; is very weak and indolent.

June 5. From this time convalescence is more rapid, and on the 10th the monkey has fully recovered. Blood planted from ear tip on 29th, 30th, and 31st gives bac. icteroides, with an accidental coccus.

June 2, a companion ape—a fine, large male—was admitted to the room and insufflated the same day. Temperature, 38°. June 3, again insufflated. June 5, again insufflated. On yesterday and to-day the animal is not lively, but dull and listless, eating less than usual. June 6, he is brighter, and from this time has shown no signs of illness. Owing to his exuberant health, it was almost impossible to get temperatures, and those taken showed no indication of illness. Result, negative.

May 31, a small Brazilian monkey was introduced into the room and insufflated a number of times without appreciable effect.

Besides these animals, we have had ready reaction to the artificial infection in cats, and the organism is infectious to goats, kine, horses, and the ass (Sanarelli). Therefore, the bacillus icteroides fulfils the third postulate of Koch, and necropsies in some animals meet the further demand of the fourth—that the organism must be found generally distributed, as in the human body dead from this disease.

INFECTION BY WAY OF THE RESPIRATORY TRACT.

By an almost common consent, at least without serious denial, the theory of the convection of the morbific agent in yellow fever by the atmospheric air has prevailed. The mass of evidence in the very voluminous literature of the disease establishes this fact, and its introduction into the body with ingested food and drink has never claimed attention. As a result of their investigation, the "board of experts" appointed by Congress for the purpose of reporting upon this disease, as it prevailed in 1878 in the South, states that "Atmospheric air is the usual medium through which the infection is received into the human system."

While this unanimity of opinion has prevailed, there have been few expressions as to the probability of the lungs being the primary seat of the disease. The theories of the earlier writers that the disease was due to "noxious emanations" and "effluvia" from certain sources also conveyed the idea that such injurious agents were absorbed from the lungs; as also the later theory that some specific organism, in its evolution *outside* of the human body, produced a toxic substance which must be absorbed through the lungs, and thus give rise to the disease. All recent observers in this field have recognized the probability that the disease was due to one of the pathogenic bacteria, but the mode of its invasion has been as obscure as the organism itself. Misled by serious and somewhat constant local manifestations in the alimentary tract, the most careful attention has been paid to this locality in the effort to isolate the causative germ, to the partial exclusion of others. And it

is to this fact mainly that we owe the very general failure of observers to identify the organism. As in enteric fever, we sought the local manifestation, and since this seemed greatest in the alimentary canal, this was supposed to be the seat of the primary colonization. But our later knowledge in enteric fever has changed this, and we now recognize that "Typhoid fever is[1] no more primarily intestinal than is smallpox primarily a cutaneous disease."

A number of cases recently collected by Bryant[2] demonstrate that the bacillus of Eberth gives rise to an intoxication from its colonization in the lungs, from which it may pass to the general circulation, identical with "typhoid" fever accompanied by intestinal lesions. In these pulmonary cases the intestinal canal appears practically normal, and the lungs may or may not show broncho-pneumonia.

Sanarelli calls attention to cases of yellow fever *without* the usual lesions of the digestive tract, and we too have observed these, i. e., that of Private Smith mentioned above, in which they were poorly marked. A close analogy has been always observed between yellow fever and typhoid, as many of the distinctive terms applied to the former will show, as typhus icteroides, etc., and it is remarkable that this is maintained in the characteristics of the two organisms, and in the mode of their invasion.

Commencing our observations with the knowledge that the general belief was that the intoxication in yellow fever arose from the intestinal canal, it is not remarkable that we also shared in this belief.

Sternberg[3] had declared that "it seems probable that this germinal principle of the disease is contained in the alvine discharges," of course meaning that it had developed in the canal after its introduction into the body.

A careful search, however, in a number of cases, inclined us to the belief that the b. icteroides was not usually found in this canal, and later to the conclusion that it was there found *only* as an incident to a hemorrhage into the canal.

Sanarelli[4] failed to isolate the bacillus from this canal.

Sternberg's[3] extensive work gave no result so far as the isolation of the "germinal principle" was involved.

So far, then, as the mode of invasion was concerned at that time the theory of Sternberg was paramount. For, although Sanarelli[4] succeeded in producing the disease by intratracheal injections in animals, he seems to have attached no importance to them more than to ordinary subcutaneous inoculations, and declares that "il est done possible que la contagion du virus amarilligène puisse s'effectuer, même dans la nature, par l'intermédiaire de l'air. Cela serait d'accord avec la plupart des opinions dominantes sur ce sujet,"[4] and that the transmission of the malady, in guinea pigs and rabbits, may be obtained experimentally, "même par les voies respiratoires."[5] Indeed, he says, that by following the method of incubating large fragments of hepatic tissue, "elle m'a permis d'établir *exactement* le *siège* et la voie de *diffusion* du b. ictéroïde,"[4] in this organ. Also "en avoir signalé la présence dans le sang circulant." In other words, Sanarelli, while excluding the idea of *localization* in the intestinal canal, assumes that once introduced there results from the presence of b. icteroides an infection of

[1] Osler, Principles and Practice of Medicine, p. 7; 1899.
[2] Br. Med. Jour. Apr. 1, 1899.
[3] Report on yellow fever, M. H. S., 1890.
[4] Annales de l'Institut Pasteur, June, 1897.
[5] Page 509, idem.

the blood, a veritable septicæmia, "*une infection du sang*," for he says, in regard to the choice of the intravenous method of inoculating dogs, that one can readily comprehend it, if one remembers that "the bacillus of the yellow fever does *not live in the tissues*, but in the blood,"[1] which is also the medium of circulating the poison which it therein produces; although in another place he states that "these microbes localize themselves in the spleen, where, during the disease, they remain without multiplying to any great extent,"[2] from which they at a given time "burst forth into the general circulation, proliferate rapidly and kill by septicæmia."[3]

To this we do not assent, nor can we find in the statements of Sanarelli anything to lead to that conclusion, for the reason that from the mode of his infections there must have in all cases resulted a "veritable septicæmia," and it is not clear to us that a condition so patent can be received as proof of the theory that this disease is primarily of the circulating blood. Moreover, the mass of proof from his work shows that the disease is temporarily *local*, even in the subcutaneous inoculations of animals, for of those animals "sacrificed" to establish the period of the general infection after such inoculations, he found this to have been established only after thirty-six hours.

Writing of the method of "*infection par les voies respiratoires*," he says that it has a certain interest, because "*il reste encore* à établir quelle est en réalité la voie de pénétration du virus dans l'organisme humain,"[4] and further declares that the bacteriologic researches in such cases are most frequently negative.

Our experiences are confirmatory of the latter statement.

Also in regard to the distribution of the organism in guinea pigs after this method of inoculation, he says that the process has less the type of an ordinary general infection than of a "veritable intoxication," which admits of the construction that he considered these cases peculiar; but he calls attention to the analogies existing in these cases and those in man in which the body is found at necropsy sterile or without the presence of b. icteroides.

In our animal experiments we have shown that white mice and guinea pigs so quickly develop a septicæmia that it has been impracticable for us to assert that the disease in these animals is primarily localized in the lungs, but we have found it impossible to produce the disease in guinea pigs by feeding them the bac. icteroides, as was done with mice.

Although the mice may be classed as ingestion experiments, the constant failure to find the organism in their intestines shows that the mode of infection was not by an ingestion, and since the organism was found in their bronchial tubules, they should be classed as inhalation experiments.

Dog A was fed upon food literally soaked in bouillon culture of b. icteroides for three weeks, with not a sign of change. The culture was then placed within the intestine beyond the very acid stomach, and gave no reaction whatever.

On March 21, 1899, two young guinea pigs were placed in a glass jar and fed upon bouillon and agar growths of b. icteroides from Montevideo until April 10, with no reaction; both pigs subsequently used to test b. icteroides intraperitoneally, and succumbed.

On March 8, 1899, a young rabbit, half grown, is injected by means

[1] L'œuvre médico-chirurgicale, April, 1898, p. 299.
[2] Idem, p. 296.
[3] Idem, p. 305.
[4] Annales de l'Institute Pasteur, June, 1897, p. 481.

of a soft rubber catheter passed into the stomach, with 5 c. c. of b. icteroides in bouillon. There was no reaction, animal gave no sign of illness, and ate freely. It was kept under constant observation until the 20th, with no results.

May 4, 1899, a half-grown guinea pig was confined in a glass jar, and fed upon bread soaked in bouillon culture b. icteroides (Smith) from this date until the 25th, when, needing the jar for other use, he was returned to the cage and watched. On the 30th he was quite well.

In this connection we introduce an experiment of placing the culture in the intestine beyond its stomach.

On May 3, 1899, a pig of 550 grams was laparotomized, a loop of the small intestine drawn out, and 0.5 c. c. of b. icteroides (Smith) introduced by means of a hypodermic syringe, the puncture point being carefully sterilized upon withdrawing the needle. Done under ether. Reaction good. May 4, animal is not bright; temperature, 38.3°; eats a little. May 5, animal is quite sick; temperature, 39.4°; eats nothing. May 6, animal is quite sick; temperature, 39.8°; dies at 3 p. m.

Necropsy (at once).—Wound nicely healed; vessels of skin and mesentery engorged; peritoneum not inflamed; fluid opalescent; liver is somewhat yellow; spleen looks normal; lungs congested; heart full of blood; kidneys congested; the urine is albuminous; the stomach contains "black vomit;" the duodenum also; cultures from organs and fluids. The blood gave a mixed culture of icteroides in one tube (with colon) and a pure culture in another; the other organs gave it in pure culture; the peritoneal fluid was mixed b. icteroides and coli communis.

In this case we are afraid that the very delicate walls of the intestine did not tolerate the injury, and that some bacilli entered the peritoneal cavity, either at the time or later from leakage through the puncture. At any rate the pig reacted precisely as did those injected intraperitoneally.

On May 11, commenced to feed two domestic pigs, 6 weeks old, native, upon food impregnated with b. icteroides, in separate compartments of an isolated cage. On this date we gave each of them in its basin 2 c. c. bouillon culture from No. 18, New Orleans, and No. 36, New Orleans, and from Smith, or 6 c. c. in all; besides several tube cultures, on agar of the same. They devour the agar cultures with avidity.

On May 14, gave 2 c. c. bouillon culture of bac. icteroides and an agar culture to each one; they appear well. On May 18, fed them two mice dead of icteroides M[1] and N. On May 19, gave each one 250 c. c. of a twenty-four-hour bouillon culture icteroides, Montevideo. On May 22, appear well; eat heartily; again given 250 c. c. bouillon culture from No. 18, New Orleans. On May 24, fed them the organs of a guinea pig dead from b. icteroides, to which also have been given full doses of Helenina as a curative. On May 25, fed them three fine agar growths each of b. icteroides from the same pig. On May 27, fed them the organs of pig No. 30 dead of b. icteroides. On May 29, fed them each two agar tube growths and 2 c. c. each fresh bouillon culture of b. icteroides from Professor Sanarelli at Bologna, Italy. They appear quite well and fat.

On June 1, the pigs are in perfect order, three weeks from date of first exposure. On this date we injected into the peritoneum of one of them 4 c. c. of a three-day bouillon culture of b. icteroides, planted from a bulb of rabbit's blood sent us by Professor Sanarelli from Bologna and marked "very virulent."

On June 2, pig has eaten all of his food; is bright and shows no

uneasiness; 5 p. m., is well and eats. On June 3, pig is quite normal; again injected into the cavity of the abdomen 5 c. c. of a twenty-four-hour bouillon culture made from the virulent blood sent us as above stated. At 5 p. m., the two pigs are alike in that everything has been eaten from their respective pans, and they cry for more. On May 4, both pigs are normal; can see no trace of illness in the injected pig.

It would appear, therefore, that the infection of yellow fever is always admitted by the respiratory tract and not by the alimentary tract. Moreover, we have shown that in rabbits and dogs, to which the infection has been conveyed by insufflating the lungs, there exists purely a local infection, without any septicæmia and that the organism can only be regained from the secretion of the lungs. In these animals the colonization is localized, the systemic invasion is of the toxins alone. As confirmatory evidence we will here introduce a number of cases which contracted the disease in our animal room, which was large, well ventilated, and kept thoroughly clean.

Rabbit S. S., pregnant female, was first noticed on the 16th of April, 1899, to be acting queerly and was isolated and given cotton wool for bedding. On the 17th she was quite wild, tearing up and remaking her bed frequently. She was found dead on the 18th at 6 a. m., having dropped six pups during the night, two alive and four dead.

Necropsy (at 9 a. m.).—Body kept on ice. No rigidity; eyes yellow and injected; the vulva bloody; inner surface of skin and the abdominal walls yellowish and much congested; on the left side there is a large intermuscular extravasation; vessels of the mesentery are engorged; there are a few small extravasations between the layers of the mesentery of small intestines; the peritoneal covering of the uterus is hemorrhagic; the liver is yellow-red in color; of nutmeg appearance, with plaques of yellow; spleen normal in size, congested; kidneys are congested; the left heart is contracted, the right is full of dark blood. The lungs were carefully examined, because we had lost a guinea pig sometime before from lobar pneumonia, and we feared that this had gotten among the animals; the lungs were bright red, well contracted, floated high in water bath, although there was much stasis; they felt generally crepitant to the fingers and gave no appearance of lobar pneumonia; a few lobules were atelectatic, a broncho-pneumonia; the mucosa of trachea and bronchi was covered with muco-pus, at points blood stained; there were numerous small extravasations; there was no pleurisy; the stomach wall had ruptured at its cardiac end; the bolus of food lying in the abdominal cavity was covered with a dense layer of mucus stained *black* in patches from extravasated blood; the mucosa was generally stained a deep wine color, and there were many small hemorrhages; small intestines the same; pericardial and peritoneal fluids were bloody; the uterus contained an embryo nearly at term; much clot and débris; its mucosa washed shows many extravasations; bladder contains less than one c. c. urine, which becomes solid when dropped into Esbach's solution. Culture tubes from liver, spleen, kidneys, blood, and feces.

Liver examined for fatty degeneration. Osmic acid shows the cells full of dark granules. Liver tissue dried over sulphuric acid, extracted with ether, and this evaporated left free fat, as follows: Fresh, 8.150 grams; dried, 2.675 grams; residue, 1.505 grams; fat, 1.170 grams, or 14.35 and 40 per cent.[1]

All tubes sterile after seventy-two hours except those made from tracheal and bronchial *mucus*. From these, after much trouble, we isolated b. icteroides in pure culture.

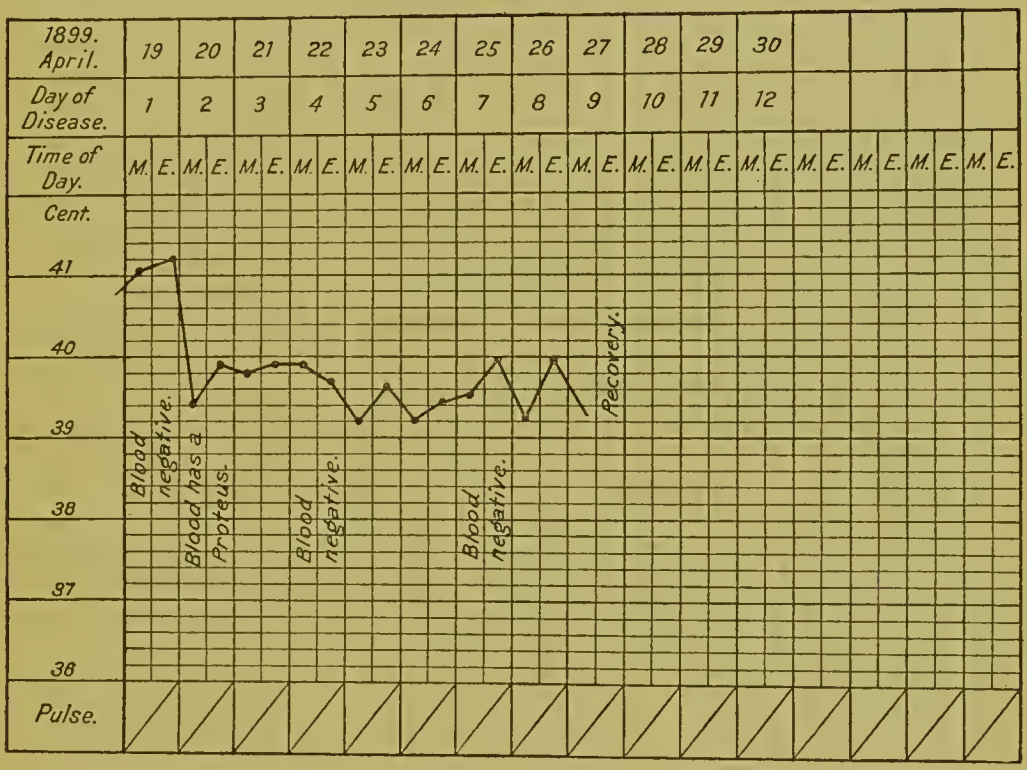
[1] Gautier gives for the fatty livers 2.5 to 17 per cent.

Necropsy was made on an embryo taken from the membranes and cultures made from blood of liver, etc., but after seventy-two hours all remained sterile. At the same time cultures were made from the body of an embryo found intra-utero, which after seventy-two hours' incubation proved sterile.

Rabbit S, a female just delivered of six pups, is noticed to be quite ill, a. m. 19th of April, neglecting her young and acting strangely; temperature, 41°; pulse and respiration very rapid; blood from ear vein planted. At 4 p. m. she is noticed picking at food, but has eaten nothing; drinks copiously; pups are nearly full term.

On 20th a. m. she is still ill; eats nothing; attends to her young but little; pulse and breathing rapid; blood from ear vein again planted; p. m., she seems quite ill. On 21st, breathing rapid; seems better; p. m., eats very little; five pups are dead—emaciated. On 22d, blood again planted.

[Rabbit S.—Disease, yellow fever.]



From the tubes first made from the blood there was obtained a proteus which liquefied gelatin on the second day—evidently a contamination accidental, since none of the other tubes were infected after seventy-two hours.

Necropsy was made on the pup which died on the 26th, and tubes planted from blood and organs, but, unfortunately, *not* from the bronchial mucus, since this was at the time not fully appreciated. After seventy-two hours there were several colonies of a colon bacillus—none of b. icteroides.

May 1, 1899, there was found dead a pup No. 1 of dog C, which pupped April 24.

Necropsy.—Dead several hours; rigidity; compound dislocation of right forearm at elbow; tissues dry; vessels full; liver congested and of a yellow color in patches. The spleen is normal; kidneys congested; lungs congested; heart in diastole; culture tubes from all organs and the blood and fluids and bronchioles.

There was, after forty-eight hours, a gross rod in the heart and peritoneal tubes, which turned orange red on agar-agar after several days. The spleen gave a pure colon growth; the liver a mixture of colon and the orange-red bacillus. The tubes from the bronchi gave freely a highly translatory rod, which proved fatal to a cat in thirty hours, with characteristic post-mortem appearances, and with the bac. icteroides in *pure* culture in the spleen of a rabbit after an intra-peritoneal injection of a bouillon culture from the cat's heart blood.

On May 17, found dead in cage, pup of dam C, born on April 24, No. 2.

Necropsy.—Body of a well-grown, fat puppy, the tissues yellowish; tongue and mouth bloody; no sign of any injury; vessels of mesentery are full; the liver is yellowish in plaques and is congested; the spleen is a little enlarged; kidneys congested; bladder had five drops *only* of urine, and this in Esbach's fluid immediately solidified; the heart in diastole; the pericardium and the pleuræ were normal, or congested; the lungs presented numerous lobules contracted in broncho-pneumonia; mucosa of trachea and large tubes ecchymotic and bathed in muco-pus. Culture tubes from heart blood, liver, spleen and kidneys, urine and fæces, also from pericardial and peritoneal fluids.

Portions of hepatic tissue, teased in 2 per cent osmic acid, showed very free degeneration of the hepatic cells, many presenting several black-stained droplets of fat. The liver gave as follows: Fresh, 4.475 grams; dried, 0.956 grams; débris, 0.662 grams; fat, 0.294 grams; or fresh, 6.35 per cent; dry, 30.75 per cent of fat extracted.

The cultures from blood and organs were sterile after seventy-two hours, except from the liver, which gave a colon. The cultures from the tracheal and bronchial mucus gave mixed cultures, from which bac. icteroides were isolated in pure culture through animals.

On May 20, found dead in cage a rabbit pup from dam No. 27, born on the 12th.

Necropsy.—A thin small animal, dead for some time; organs much decomposed; liver is almost black; the stomach walls show port-wine color and submucous extravasations; the intestinal mucosa deeply congested; spleen normal in size; bladder empty; lungs congested, broncho-pneumonic, and float deep in water bath. Cultures from organs and fluids give a colon-mixed growth; no icteroides.

The necropsies of three white mice, X, XX, and XXX, are as follows:

Necropsy mouse XXX.—Dead several hours. Skin is yellowish on inner surface; vessels are distended; liver is very yellow from fatty change; spleen appears normal in size; urine is albuminous; vulva is swollen, and there escapes a bloody discharge; the uterus contains five embryos, possibly of mid term; one has been aborted. Cultures taken from organs and fluids, bronchial tubes, and from a fœtus. From the fœtus a pure colony of b. coli communis was obtained, similar to the "X" bacillus of Sternberg. The b. icteroides was not isolated from the other tubes.

Mouse X.—Found dead in general cage, May 8, 1899. Dead for some time; much decomposed; liver is a mottled yellow-red; spleen is normal to the eye; abdominal contents decomposed. Cultures from all organs and fluids and from tracheal mucus. All tubes gave the presence of a suggestively motile rod, but we were not able to isolate bac. icteroides.

Mouse XX.—Found dead in cage; the liver is distinctly fatty; spleen normal; stomach and duodenum hemorrhagic; cultures from blood, liver, spleen, and fæces. The bac. icteroides isolated in pure culture from blood and organs but not from fæces.

On May 25 a large pregnant guinea pig was noticed to be droopy and not eating; this was attributed to her condition; on the 26th and 27th she ate a little soaked bread, but was very quiet. On the 28th she brightened up, but died at 3 p. m. of 29th. Temperature 36.5°, or in collapse.

Necropsy (*at once*).—Body of a fine large pig; nose and lips bluish; black bloody discharge just commences from vulva; skin vessels full and there is slight icterus of inner surface of the pelt; the uterine walls are dark red; the bladder contains 3–5 drops urine that proves albuminous in Esbach's fluid; vessels of mesentery full; liver is dark from congestion, but the thin edges are yellowish; gall bladder full; spleen much enlarged and friable; kidneys swollen; right heart distended; pericardium and pleuræ ecchymotic; the lungs generally congested; the middle right lobe is *lobularly* pneumonic, as also the lower left lobe; bronchial mucosa inflamed; the stomach contains undigested food; the mucosa is very slightly congested; that of the small intestine also, which contains much bloody mucus; cultures from all sources—from mother and from amniotic fluid and from pups, of which there are six in utero.

Liver tissue teased in osmic acid gives no appreciable fatty change, unless a few minute dark granules mark the commencement of this change in the albuminoids of the cell. From the cultures of the amniotic fluid and the embryos there were pure cultures of a primarily translatory bacillus, which resembled bac. "X" in all respects, even to the subsequent loss of its motility. The liver gave a colon; the others were sterile, except those from the bronchial mucus, which undoubtedly contained an organism resembling icteroides in mixed culture, but we were unable to obtain it pure.

In this connection we must call attention to the fact that in our animal room, which was large, well lighted and well ventilated, and well cared for, there had been but *one natural death* among our animals, which were kept in galvanized wire cages, from December 1, 1898, to April 7, 1899. This one death was caused from the pneumococcus, isolated post mortem from lobar consolidation, during February.

On April 7, 1899, we made our *first* insufflation in the room adjoining and connecting with the animal room. We were as circumspect as possible in insufflating the dog A, and did not anticipate any casualties among the animals. This was done on the 7th, the germ being evidently borne by the air into this room, for rabbit SS died during the night of the 17th, or just ten days from this *air-born exposure* (although animals had been *injected freely* and had died in this room for several months). If three days are given in which to have caught the infection, the balance of seven days is just the cyclic period of this disease in this animal, as it is in guinea pigs inoculated with minute doses of the bacillus, *thus* avoiding a primarily fatal intoxication from preexisting toxins; and as in dog A, in white mice, and in the monkey. From this animal's bronchial tubes was regained the bacillus icteroides, never found by us save in those sick or dead from clinical yellow fever. This animal gave 40 per cent of *fat* in the dried liver, and 14.3 per cent of the entire weight of the fresh liver.

Coincidently, another female rabbit, S, under like conditions becomes very ill, does not care for her young (*all* of which finally die), but recovers in seven days, or on the 26th, having fallen ill on the 19th.

Again, mouse XXX is found in miscarriage and dies with five pups in utero.

A large guinea pig dies just before miscarriage, which seemed immi-

nent, in five days from first observation. In these *nine* cases the specific agent of infection, the b. icteroides, was found in rabbit SS, mouse XX, and from two puppies, or four of the nine. Further, in three of these it was isolated from the bronchial mucus, *all other tubes remaining sterile*, the mouse alone showing septicæmia.

Moreover, rabbit S should be eliminated from the contrary cases, because she *recovered*, and since there is *never a septicæmia* in these animals (see No. 15 and No. 25), we, of course, could not obtain the bronchial mucus from which to attempt the isolation of the bacillus. This elimination made, we will have a result of 50 per cent of isolations of the bacillus from these cases, or nearly as good as that of Professor Sanarelli in his series of cases of yellow fever.

Since these facts establish the mode of entrance and the seat of the primary disease, the changes which take place in the lungs become a matter of great interest. Many of the earlier authors, as collected by La Roche,[1] make little mention of clinical features that could have been attributed to these organs, yet many of them describe "an embarrassment of the respiratory act," a "laborious respiration," a "sense of inability to expand the chest and inflate the lungs," none of these symptoms being considered "*constant* attendants," and Rush gives among premonitory signs of the disease "a dull pain in the side" (chest), or "a hoarseness or slight sore throat." Hemorrhage sometimes takes place from the lung. Since our attention has been called to this point we have had access to four undoubted cases of yellow fever, namely, Everette, Sesein, Quintela Macias, and Rodriguez, and in three there was clinical evidence of lung trouble, such as râles, some pain, and hemoptysis; in one, Everette, this was an alarming symptom; in one, Kauna Sesein, there was no respiratory difficulty. In our personal experience we can recall other cases in which there was broncho pneumonia. In necropsy work the lungs have been relegated to an undeserved obscurity, but nothing is more frequent than to find these organs the seat of an extensive broncho-pneumonia, or a condition resembling closely the "grippe lung," in which the principal change is that of the loss of elasticity in the frame work, due indirectly to the influence of the toxin, which primarily affects the vasomotor control of the larger blood vessels, the stasis in the capillaries that results still further influencing the elastic frame work of the alveoli, which is their sole support. The influence of the b. icteroides thus localized is then similar to that of the Pfeiffer bacillus in that they do not, as the pneumo-bacillus, give rise to a complete inflammation, or the congregation of leucocytes and formation of a fibrinous deposit. There is less local change in the invasion of yellow fever than in grippe. And the experimental work in animals shows this, that the local influences of the injected organism, b. icteroides, are very slight. Peritonitis or other serous inflammation is the rare exception, and in the connective tissues there is but little change other than the intense congestion, which is general. The toxic influence upon the tissue of the lung explains, even in the absence of cough and pain, that feeling of pulmonary insufficiency described by La Roche, as well as that omnipresent symptom, which is universally recognized, the dusky, bluish condition of the skin, due to congestion of the vessels and diminished aëration of the blood. In the blood itself there seems remarkably little change in the elements, for in a number of cases in which we counted the cell elements these seemed normal, save for the reduction of the hemoglobin. We believe, therefore, that it is from the absence of *special* local reactions in the lungs

[1] On yellow fever, vol. 1, p. 132.

that we have been led to misinterpret their importance in this disease, and it is a matter of regret that we did not realize this fact at an earlier date.

From three cases—those of Kauna Sesein, Quintela Macias, and Rodriguez—we examined the sputum most carefully, with the result that the b. icteroides was isolated from one—K. Sesein. In the two others it was not isolated pure. However, we are informed by Dr. Meacham that he isolated "from the lung" of Q. Macias a bacillus which he thought to be icteroides.

The isolation of this rod from the sputum promises to be most difficult, as we have found the sputum rich in micro-organisms, many of them of the colon group, and in one case—Quintela Macias—the "X" bacillus was obtained in pure culture and in beautiful activity. We were led to the plan of adding a little bouillon to the sputum, triturating it, and placing for twenty-four hours in the ice chamber, the motile organisms responding quickly, others more slowly at 18°, plates from this mixture giving an absence of many of the ordinary germs of the air encountered otherwise. The sputum, therefore, is of a diagnostic value, and this will be the greater in those cases of the disease in which there may be no secondary invasion of the blood, but in all before this invasion takes place.

SECONDARY INVASION.

The primary reaction in yellow fever, lasting from "three to five days" as the rule, is followed by a more or less distinct remission of temperature, subsidence of all other symptoms, and an apparent improvement of the patient, either to be continued into convalescence or, after a "period of calm," to give place to another rise of temperature and a notable aggravation of all symptoms. This latter access of the disease is generally known as the "reactionary fever," or, as Bancroft termed it, "the second paroxysm."

It is one of the most prominent of the clinical features of this disease, and among clinicians of endemic or epidemic centers it is considered of the greatest assistance in formulating an opinion. So true is this that the most experienced of these clinicians prefer to defer an opinion until there is no longer doubt as to its occurrence. From our earliest professional recollection, in a possible epidemic area, the most intense interest has centered around this "second paroxysm," and we have heard from the lips of many the expression of an utter noncomprehension of this clinical phenomenon, which embraces the period of calm or cessation of fever. This characteristic clinical peculiarity of yellow fever, stated by La Roche[1] to be "of itself almost sufficient to enable us to diagnose the case," occurs in perhaps a majority of all cases, the same author describing its absence in those cases of a mild type "in which symptoms of the first stage alone are noticed," or "in the violent and congestive forms of the disease," which terminate before these later symptoms develop. This corresponds with the classification adopted by Sternberg,[2] of "mild yellow fever," "grave yellow fever," and "yellow fever siderante," there being numbers of cases merging from the first into the second. The third variety is distinct, fulminant, foudroyante, fatal.

We have isolated the cause of this infection, and have, we trust, shown its natural pathogenicity to animals and the mode of its invasion. We believe that there is sufficient anatomic and bacteriologic evidence to warrant these opinions, and we ask that in this matter, which has

[1] La Roche on yellow fever, vol. 1, p. 428.
[2] Report on Y. F., 1890., Treas. Dept.; Document 1328, Marine-Hospital Service.

exercised so many observers who have preceded us, that the errors of judgment which may be expressed be attributed only to that one of us[1] who has had in control all experiments relating to natural infection, invasion, and allied subjects. There is no adequate explanation advanced to account for these clinical facts, as they have been accepted for years. The studies in histo-pathology have resulted in no elucidation of them.

These facts, in a moderately severe case terminating in recovery, as in the second class of cases, are a distinct access of temperature, reaching the maximum early in the disease, followed by a no less distinct fall to the normal or below it; this in turn followed by a quiescent stage, to be succeeded by a condition more aggravated than the first stage, and in which increase of temperature, remittent or intermittent, may or may not be a feature. The time occupied by the first stage may be from two to five days, or longer; that of the second may be from a few hours to one or two days; that of the third is very irregular and may be terminated by death in one day, or extend into a tardy convalescence. Although pathologically we have no explanation, we believe that there is an explanation to be based upon bacteriologic data.

The first stage is the result of the colonization of the bac. icteroides, the intoxication producing the rise of temperature and other symptoms; this lasts from two to five days. During this time, our experience has been, the blood is free from the organism for the first day or two; in other words, it is strictly localized in the pulmonary tissues, and is found in quantity in the blood only after several days.

The first invasion in the pulmonary tissue followed by its intoxication soon gives place to an improvement, which frequently, and in some epidemics almost invariably, is the commencement of convalescence. During the earlier part of this time the blood is sterile, but its contamination takes place about this time, from the second to the fifth day, after which all symptoms become aggravated—by *what? By the secondary invasion of the blood by the b. icteroides from the lungs, the primary seat of the colonization.* This corresponds with the known incubative period of the disease, for, if the changes in the pulmonary tissue, dependent upon the first colonization, allow the entrance of the germ into the general circulation upon the first or second day of the disease, the minimum period of incubation of the organism in the blood (formation of the second colony) would not permit it to be perceived until the second, or third to the fifth day, thus giving a clinical picture of an access of temperature (after three days' incubation in the lungs) for from two to five days, then a depression of one to two, to be closed by an irregular condition of fever, from septicæmia.

The thermo-charts of our animal cases give no pictures of this more or less abrupt decline in the temperature; their fevers are typically of *one* paroxysm, because in them there was no subsequent complicating invasion of their blood.

The exception to this statement was in the instance of the disease in the ape, in whose blood the germ was found after the third day. We do not believe that this perfect picture is frequently seen at the bedside, and this has been observed by others, for there exists the "impossibility to establish in yellow fever a '*type thermique specifique.*'"[2] And this irregular expression of this well-established fact is due to irregularities of incubation and secondary invasion, in turn due to less virulence of the microorganism, or greater resistance of the individual attacked. We assert that this is reasonable, for this secondary invasion or "third

[1] Wasdin. [2] Sanarelli.

stage," or "second paroxysm," is quite as *specific* as the primary or first paroxysm; therefore, it must depend on equally as specific a cause, and we see the analogy between this increase of severity of the symptoms of this secondary invasion of the germ into the blood and the well-known severity of symptoms which follow the *intravenous* injection of the virulent culture into the horse during the process of antitoxin production, although the animal has been prepared for this septicæmia by preceding intoxication with filtered cultures or toxins. We therefore submit that the disease is one of two paroxysms whenever there results from the first colonization in the lungs a second one in the blood—a septicæmia.

In the mild cases yellow fever is purely local and of one simple paroxysm, due to an amount of intoxication against which the system readily contends. The rare *siderante* cases are those in which the virulence of the organism plus a lessened individual resistance results in the production of an intoxication quickly fatal. In these cases there either is not a blood invasion or a too limited time for an incubation sufficient to assist in the fatal result. This corresponds with our bacteriologic results obtained post-mortem. Case No. 11 was of this kind, ending fatally in fifty-three hours, in which the blood was almost destitute of bac. icteroides, and Sanarelli refers to these cases "where the cadaver is almost sterile, and where the quantity of urea contained in the blood is very high," or "where the cadaver gives cultures almost pure of other microbes than the specific one," in which "one may attribute death to a septicæmia" secondary in the course of the disease.

In these fulminant cases whatever the increase of urea in the blood this depends upon the changes induced in the liver by the toxin, and is only a contributory cause of death. In those developing a colon, or other septicæmia, even in pure type we question the absence of the specific organism, and we do this upon the basis of our larger percentage of isolations of the organism than that of Sanarelli. Moreover, his assertion of the antagonism existing between bac. icteroides and b. coli communis and the streptococci is not sufficient to account for their absence from the cadaver or living blood. Our experiments with the toxins will show that this position was not well taken, for the only toxin at all fatal to bac. icteroides was that of its own production. This fact might explain the absence of the bacillus from some dead bodies, as in the siderante cases, in which there must be an intense intoxication, or their presence in very small numbers, but it would also then apply to all dead bodies, in many of which, however, we find the organism.

How much influence the presence in the blood of b. coli communis, b. proteus, or the micrococci may exercise upon the termination of a case we are not prepared to assume, but we have no doubt that these auto-infections add materially to the mortality of the disease, although our experience leads us to believe that they do but little harm in comparison to the more potent organism, b. icteroides, and that this harm will depend upon the presence principally of one of the more virulent forms of bac. coli communis, whether it enters the blood with b. icteroides from the lung or during the "last hours of life" from the changed intestinal tract. We can not accept the theory of Sanarelli that these facts are to be explained by the "location" of the bacillus in the spleen after its entrance into the body, its keeping dark "during the entire course of the malady," and finally, at a given time, bursting out upon the blood, invading the whole body. In lieu of this most unsatisfactory explanation we offer the above solution of the vexed problem of yellow fever.

THE DISEASE IS NOT PRIMARILY A SEPTICÆMIA.

The admiration which we naturally felt for one so learned and heroic as Professor Sanarelli at the commencement of our investigation has, during its continuance, but given place to a greater one, and we must acknowledge the greatest amount of assistance from his published memoirs, as well as the most lively appreciation of his written expressions of regard for us, and his helpful suggestions to us. Therefore it is with a feeling of the greatest reluctance that we differ from him in these minor matters. Still from a therapeutic point of view it is a matter of much importance that we should know the seat of the specific agent in these infections, in order to attempt to combat them locally, thus limiting their toxin production. In many cases nature does this effectually, the disease being limited to an ephemera, or as McFarland, of New Orleans, phrased it, "resembles nothing *more* than a violent cold." Such cases are the most usual in children, giving rise to the expression by Guiteras that yellow fever is one of the most innocuous diseases of childhood. Why this should be so we do not comprehend, yet we know that the physiologic processes in children, the tissue metabolisms, are very active, and perhaps these are sufficient, on the invasion of the lungs by bac. icteroides, to limit the colonization to that locality, and to prohibit a septicæmia. Clinically, too, we would expect graver symptoms, and more frequently fatal results, were these cases septicæmia produced by so virulent an organism. Rabbits seem to possess in a great measure this power of keeping the germ localized, for only in number 15 did we find an invasion of other tissues, and in this case there was no septicæmia. Dogs possess this localizing power to a perfect degree, and this is in accord with the discovery of Nuttall of the germicidal influence of their blood serum, a fact demonstrated by the difficulty with which Sanarelli and others have produced a septicæmia, by intravenous injection, sufficient to kill, and the evidence of a diminution of the numbers of the bacilli which followed these injections.

In monkeys there seems to be a lack of this controlling power, for in our single, but perfectly marked infection, the organism invaded the blood as in man.

The question naturally arises here as to the frequency of the exercise of this controlling influence in adults; as to how many proportionately are "mild cases," and how many of the class characterized by this "second paroxysm," or secondary invasion, or septicæmia? Children native to endemic localities have long been thought immune to the disease; so also, for many years in the South, the negro race; also, there has existed the custom among "yellow fever experts" to search health records for data immediately preceding an outbreak, from which to form an opinion as to the time of its inception, and the person with whom it commenced. All of which goes to show that there has never existed any perfection in diagnosis, and we do not believe that any symptoms in these very mild cases will lead us to their diagnosis with unerring certainty. We have found the bac. icteroides in the sputum of but one case of three examined for it. In one of the others it was said to have been found by another observer.

In 50 per cent of animals naturally infected, or without our intervention, it was found in the secretion from the bronchi, and in all of those into which it was insufflated. Therefore, an early examination of this secretion may result in an early diagnosis of the disease. Just here we would allude to the very remarkable degree of infectiveness of b.

icteroides, and to suggest that were these cases septicæmiæ, in which the germ is locked within the fastnesses of the spleen and in the circulating blood, they would be innocuous until they became of such severity that breakage of capillaries would result in hemorrhage, either into the lungs, intestine, or kidneys, through which the germ would again be in position to invade other persons. This is not the case. A mild case may prove as dangerous to others as a severe one, by infecting the domicile, and unless hemorrhage frees the bacillus from its septicæmic environment it must gain access to the domicile, to the environment, through some other medium. We believe this to be the sputum, or secretion of bronchial mucus bearing a portion of the lung colony.

Expectoration is with difficulty controlled in the most perfectly regulated sick room, and especial care would be required to insure cleanliness. Such contamination would prove innocuous until such time as the bacilli would be liberated by the desiccation and disintegration of the particles of sputum. Thereafter this would depend upon the opportunity of the germ to invade the lung of one susceptible to its influence.

This period has been termed the period of "extrinsic incubation," and has been thought to cover that time taken by the germ to undergo some occult change, dependent upon we do not know what, but by which it, having been reduced in some way in power, again becomes sufficiently virulent to give rise to the disease. This period has been variously stated at from five to ten days, and some observations seem to fix it at fourteen days. While we do not understand the nature of the causes which result in the pseudobacilli among the pathogens, we know that some organisms are reduced in virility by their passage through certain animals, and it may be possible that this is the case in man infected with b. icteroides, and that the germ requires a growth under such extrinsic conditions to regain its virulence. Still this does not apply to the organism taken artificially from the blood or organs and planted on the various culture media, for under these circumstances it is most virulent, and we have never seen the pseudobacillus icteroides. Moreover, the human inoculations of Professor Sanarelli were done with sterilized cultures of the organism which had been taken from the dead body, and these demonstrated the virulence of the toxin produced by their growth.

To us, therefore, it seems that this theory of extrinsic incubation had best give place to a safer one, as follows:

That the time necessary for an apartment, or domicile, to become dangerous to others after the entrance into it of one ill with yellow fever depends upon the facility with which the apartment, or domicile, becomes contaminated with the bronchial secretion of the patient and the rapidity with which this contaminating material may dry, become disintegrated by sweeping, dusting, etc., and floating in the air, may enter the respiratory tract of one entering the premises.

We have already alluded to the statement of Professor Sanarelli, that there are three classes of necropsy cases of yellow fever. The first presenting numbers of the specific organism present in blood and tissues; the second when they are not present, but in their place other contaminating germs; and third, cases that are sterile as to the blood and most of the tissues. Therefore, in a large proportion of his cases he assents to the absence of a specific septicæmia, or in 46 per cent of those he examined. One of us (Dr. Geddings) isolated b. icteroides from the necropsy cases from New Orleans, in 83.3 per cent of the cases,

still we do not believe an even per cent would prove more than that the cases were septicæmic at death.

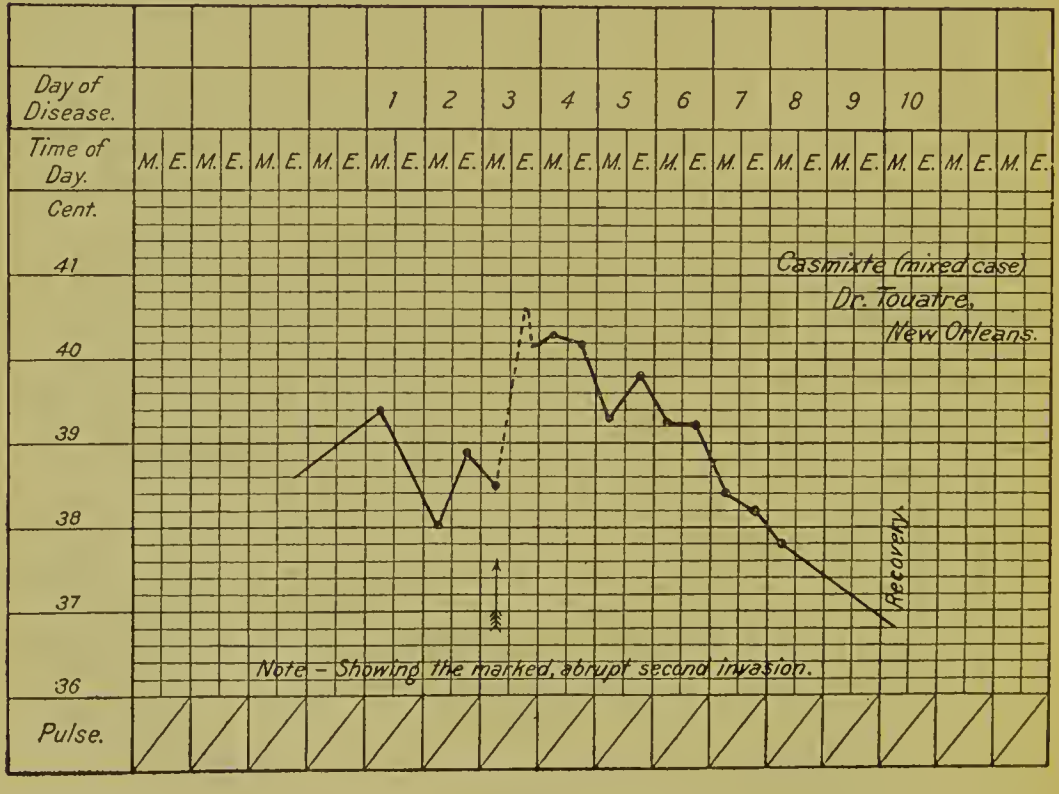
Also Sanarelli found in some of these cases the germ in the living blood, but his work did not result in so many positive isolations from this tissue as our own, yet these can only prove the septicæmia at the time the bleedings were made.

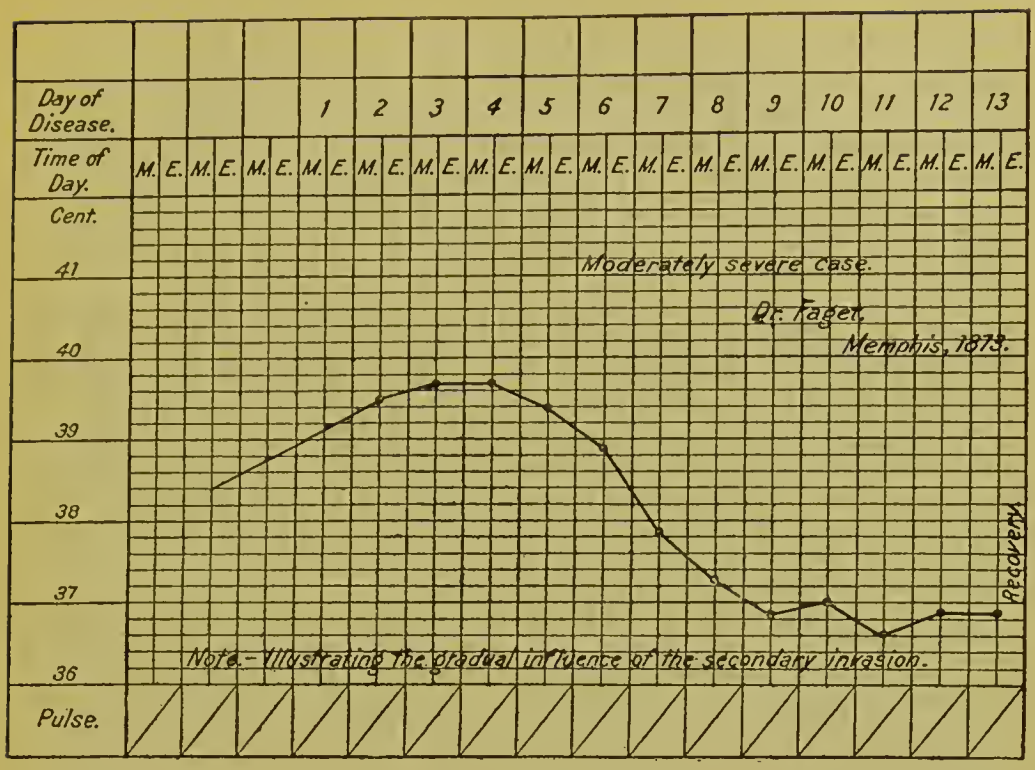
Therefore, while we realize the possible analogy between the accumulation of the malarial plasmodium in the spleen and the deeper structures, at times being entirely absent from the surface circulation, as well as the temporary retirement of the embryos of the filariæ from the superficial vessels, and the conduct of seclusion, advanced by Professor Sanarelli, in explanation of the absence of the specific organism in the circulation and its retirement to the spleen, we can not assent to it, and refer to the explanation given above.

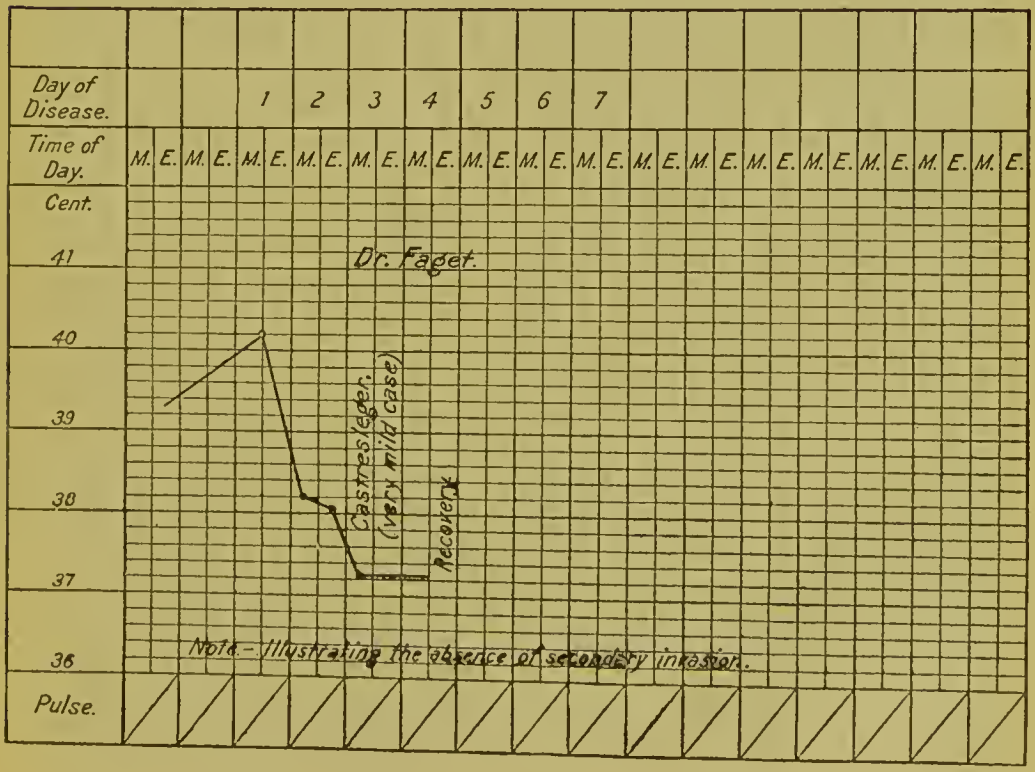
We introduce three thermo-charts illustrating the temperature tracing in the three classes of cases diagnosed by those eminent clinicians, Drs. Faget and Touartre, of New Orleans, La. (The "notes" are our own.)

These are taken from the former's "Monographie sur le type et la spécificité de la fievre jaune," 1875, in which, besides establishing his well-known law of the noncorrelation of pulse and temperature in this disease, he furnishes much valuable information.

Among other points he insists upon the *difference* between the *period* of *defervescence* in yellow fever and that of other acute infections, such as pneumonia and smallpox, determining that it is *specifically so;* that is, that it depends upon the specific cause of the disease. Some of his charts also show that the secondary invasion, then as now, may end in fatal intoxication without appreciable rise of temperature, death taking place in an algid state.




Day of Disease.
1 2 3 4 5 6 7 8 9 10 11 12 13
Time of Day.
M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E.
Cent.
41
40
39
38
37
36
Pulse.
Moderately severe case.
Dr. Faget,
Memphis, 1873.
Recovery
Note.- Illustrating the gradual influence of the secondary invasion.


Day of Disease.
1 2 3 4 5 6 7
Time of Day.
M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E.
Cent.
41
40
39
38
37
36
Pulse.
Dr. Faget.
Castres Leger.
(Very mild case)
Recovery
Note.- Illustrating the absence of secondary invasion.

THE PRESENCE OF THE ORGANISM IN THE ALIMENTARY CANAL.

"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.

There is no evidence that yellow fever is propagated by contamination of the supply of drinking water, as frequently and probably usually 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 a vessel with the disease on board before the cases of local origin occur, and these cases occur in the immediate vicinity of the imported case or vessel carrying the contagion. 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 healthy, while another, usually the most filthy, 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 the privy-vaults or upon the soil until it has been disinfected. At all events, I think it would be very unfortunate if, upon the strength of Sanarelli's negative researches, sanitarians should neglect to disinfect the *excreta* of yellow-fever patients."

We quote these remarks from the address of Dr. Sternberg before the American Public Health Association at its meeting in 1897, to define his position in regard to the seat of the colonization of this infectious organism. As before remarked, bacteriologists have been accustomed to seek the cause at the seat of effect, and in many of our known infections this has been sufficient, the "false membrane" yielding the bac. diphtheriæ, and further search is unavailing; but if the local reaction in diphtheria was less pronounced and the seat of it less prominent we can not say that the bacillus would have even now been isolated. In other words, its prominent seat contributed to the bacteriologic result. Not so, however, in yellow fever, for although this experienced observer carefully examined some forty-three dead bodies, paying more than especial attention to the alimentary tract, in which seemed the greatest damage from the disease, he failed to find any organism possessing more than pathogenic influence upon man or animals when injected into their tissues, most frequently their blood.

There is no recorded experiment, either of Sternberg or Sanarelli, showing that they realized that the organism which they claimed or supposed might be the cause of yellow fever must be demonstrated as producing the disease *under natural conditions;* that no *artificial infection* should be taken to prove a natural fact.

A realization of the importance of establishing a natural infection with b. icteroides would have induced Professor Sanarelli to have insufflated his human subjects in lieu of intoxicating them through the veins, for, as expressed by Novy, "we know nothing about the results in man following intravenous injections of the filtered products of even

the most common bacteria. What would be the results in man following intravenous injections of micrococcus prodigiosus, colon bacillus, Eberth bacillus, etc.? In all probability such injections would bring about very serious results, especially in previously diseased individuals." One natural infection in one of his insane subjects would have been far more convincing than a thousand intoxications, or artificial infections.

Bacillus "X" was constantly found in the alimentary canal of animals and man, both sick and well. But the bac. icteroides was not present in the canal of animals or man ill with yellow fever unless in the presence of a hemorrhage into the canal; and in one case with *bloody* urine, we isolated the organism from this secretion. Both bac. "X" and bac. icteroides were isolated from the sputum. Our work in the isolation of the bacillus icteroides indicates plainly that the bac. icteroides does not primarily colonize and produce its toxin in the alimentary canal, and if the bacillus is found therein it is incidental to hemorrhage. Still we would reiterate the warning of Dr. Sternberg that sanitarians should not relax their efforts to accomplish the disinfection of the dejecta from yellow-fever patients, since these hemorrhages, though very minute, are prone to occur in even moderate cases of the disease. In regard to the etiologic relation of the bacillus "X" to yellow fever, we must state, that although fully convinced of its position in the group of colon organisms by our association with it in New Orleans in 1897, we did not deem it our province to discard it from the list of possible etiologic factors until we had subjected it to the test of natural infection stated above. It has proved in our hands the most virulent colon when introduced into animals artificially, giving rise to anatomic changes very similar to those of b. icteroides. As to the cyclic period of the disease in animals under artificial conditions we must acknowledge that both b. icteroides and bac. "X" seem to exercise an influence proportionate to the amount of material injected, the fatal termination ensuing in twenty-four hours, or at the end of six to eight days. In other words, no determination as to a cyclic period can be based upon the published artificial experimental work. We therefore believe that the normal denizens of the alimentary canal have no place among the primary factors of yellow fever, however much changes in its vessels and mucosæ may exercise us in the progress of the disease.

BACILLUS ICTEROIDES NOT FOUND ASSOCIATED WITH OTHER DISEASES.

The first postulate of Koch would be unnecessarily amended if we should still further demand that the organism found constantly associated with a specific disease should not be found associated with any other specific disease in frequent instances, either prior to or after death. Ordinarily this would scarcely be considered necessary, yet in the disease in question the claim advanced by Dr. George M. Sternberg[1] that the bacillus "X," isolated from cadavers by him in Havana, Cuba, in 1889, and at that time suggested as the possible cause of yellow fever, is identical with the Sanarelli germ, and that his bacillus should receive equal recognition, has made it incumbent upon us to carefully observe whether bac. "X" is usually associated with b. icteroides in yellow fever, as well as whether it or b. icteroides is usually found associated with other diseases.

[1] Journal Am. Pub. Health Assn., October, 1898.

To this end a number of cases of disease other than yellow fever were examined bacteriologically.

No. 1.—On December 21, 1898, we saw at the Quinta del Rey a Norwegian seaman in whose case there seemed some doubt of diagnosis. History of malarial exposure and of an irregular type of fever for four weeks. When seen there was no evidence of yellow fever, and from the condition and history we thought it malarial cachexia. Careful search was not made for the plasmodium malariæ. Blood planted in bouillon; after forty-eight hours' incubation there was no growth upon the tubes, all remained sterile. Man died later; no necropsy.

No. 2.—Blood taken from the ear tip of a case of malarial cachexia, with peri-hepatic abscess, gave no growths after forty-eight hours' incubation. Pus from abscess gave b. proteus fluorescens. On December 23 we saw, in courtesy, a young girl moribund from what is here called "la fiebre de borras," and which is alone diagnosed among the native infantile population, but at times young adults, as in this case, are attacked. We were told by her attendants that she had suffered from light malarial attacks for some time, but that this pronounced attack had occurred five days before we saw her, with chill and high fever; much pain in head and limbs; nausea and vomiting, this last having been a constant and distressing symptom. There was no temperature record, but the statement that it had risen to 40° was made. On the second day there was scantiness of urine, which continued during third, but on fourth this secretion was increased, and now there was sufficient limpid or brown urine with no albumen.

Status presens: Facies dull; eyes suffused and staring, pupils dilated; gums normal in appearance; tongue pointed and red at edges; much facial subsultus, and of the tendons of the forearms; skin of face deeply congested, and that of the chest and arms; the room was darkened, and we could see no yellow tint; mentality much impaired; died seven hours later; blood plantings gave a b. coli communis and a tetrad from the skin.

A native child, with the history of having been removed to the United States during the "insurrection," or for two years, was returned to Havana in January of 1899, and after two months, or March 5, was taken ill with continued fever of peculiar type. The diagnosis of yellow fever was first made, and the fever running a too lengthened course, the blood was examined and the malarial parasite was found.

The fever occurred in distinct paroxysms with remissions. During the febrile rise there was albuminuria with much hemoglobine, the secretion being almost black. This passed off with the fever, and during the remission the albumen was barely a trace and the urine clearer. This was repeated each day, the hemoglobinuria appearing as the febrile accompaniment of the forenoon. When we were asked to see the child all suspicion of either yellow fever or of borras fever had passed away, but blood was planted, as usual, and from it a b. coli communis and a b. proteus vulgaris were isolated. Boy recovered.

No. 5.—Diego Lazaro, 39 years of age, from Spain; nine years in Cuba, most of the time in the interior; entered the city in December, 1898, and was engaged in work upon the city sewers when taken ill in March with a sharp chill, followed by high fever, much pain in head and constant nausea; this had continued for two days when he was seen, through courtesy.

Status presens: Facies anxious; eyes and skin a little yellowish; little congestion of skin; much cough and pain in chest, the expectora-

tion bloody; liver and spleen enlarged and tender; stomach tender; urine contains 20 per cent moist albumen. Blood examined for malarial organisms without finding them. There was a most marked change in the cellular elements, there being many gigantoblasts, as well as microcytes; the normoblasts were markedly deformed; dumb-bell shapes and other most irregular forms were seen, a true poikilocytosis; the multinuclear leucocytes were in excess, but not so much so as to indicate lobar pneumonia. Blood taken and cultures gave only a proteus bacillus. He recovered.

No. 6.—John Tabor, of Santo Domingo, aged 47, has had gastro-intestinal troubles for the past five years.

Status presens: Skin is pale and muddy and dry; abdomen swollen and tympanitic with thin walls; intestine irritable; there is borborygmus; at times violent diarrhea is followed by bloody stools. Emaciation is progressive; appetite poor; tongue dry and devoid of epithelium. Culture tubes, inoculated with blood from the ear tip, remained, after four days, sterile.

No. 7.—Fulgencia Ramos, aged 20 years, a native of Havana, has been sick one and one-half years. His illness commenced with a malarial attack, was followed by chronic diarrhea, and he is tubereulous. There are frequent and severe attacks of diarrhea, profuse night sweats, and hectic fever. Blood taken from ear tip in bulbs and sown on tubes of agar and bouillon remains permanently sterile.

No. 8.—Saturnino Ripa, from Spain, about 45 years old, has been sick one year. Has always drunk to excess; has suffered from atonic dyspepsia, and attacks of "bilious diarrhea," which is now constant and painful. There is œdema of feet and legs and hands; had yellow fever eighteen years ago, just after arriving from Spain. Ear-tip blood proved sterile on all media.

These cases, though few in number, were representative of the diseases prevalent in Havana, and if bac. icteroides be considered an element of "secondary infection" in yellow fever, it is peculiar that it was not encountered in some of these cases.

Also for the same purpose there were made a number of necropsies upon bodies dead of the diseases incident to Havana, equal or in excess of the cases of yellow fever examined for the presence of b. icteroides, and cultures prepared from all the fluids and organs of the body, with the result that in not a single case thus examined was there found an organism even remotely resembling the b. icteroides, the nearest being that isolated from necropsy No. 6, which proved a pseudo-typhoid organism, producing no fermentation in sugars, no indol, no acid reaction, and failing in pathogenicity in animals. It is unnecessary to inform you that this control necropsy work was done with the same care and vigor as that of the isolation of the bacillus in cases of diagnosed yellow fever, for you will recall the skepticism as to the specificity of the b. icteroides with which the one of us, to do this portion of the work, returned to Havana at the close of the Spanish-American war and resumed the investigation.

Section was made some thirty times in these cases, many of them at the city morgue, upon unknown and undiagnosed conditions. In all cases section was made carefully and the cultures made from the heart and liver and spleen and kidneys, and in many instances from the bile and urine and feces, directly upon slanted agar and into bouillon. Our experience had been in New Orleans that the practice of incubating portions of the organs gave less satisfactory results than the plantings

at the necropsy table and was abandoned. From these cases we carefully plated all cultures giving the slightest indication of the presence of bacilli comparable to b. icteroides.

The organisms found were in great variety, the b. coli communis in many forms being the most frequent and evenly distributed among the organs, an evidence of invasion during the last hours of life; bac. proteus vulgaris was the next most frequently found; then bac. pyocyaneous; staphylococcus, and least frequently (in one case) a streptococcus. In no case was an organism isolated corresponding to that of Sanarelli.

No. 6.—Necropsy held at the hospital "Alphonso Treee," February 22, 1899. Manuel Fajada Dominguez, aged 23; history of malaria. Body of adult male; marked rigor; dead eight hours; much hypostasis about the dependent portions of trunk; skin yellow in hue; pupils dilated; eyes yellow; section showed engorged mesentery; a bronze-yellow liver, much congested; enlarged, nearly diffluent spleen; kidneys normal; stomach content free from blood; mucosa clear; that of duodenum the same; bladder contains a little urine, slightly albuminous antemortem.

Cultures from blood, organs, and fluids. After forty-eight hours all tubes from blood and spleen show a mixture of motile and immotile rods, easily plated out as coli communis, and proteus viridens, and one b. violaceus.

On the 26th of February we found on a tube from the liver a slowly growing colony of a translatory rod, which was carefully studied, with the following characteristics:

Morphology; a rounded rod; 1—1.5 × .5 μ; staining readily, but not by Gram. Actively motile, with a defined steady movement. Grows well on all media at 37°. On agar the young colonies resemble those of b. typhosus, the older growth being clear and transparent, slightly iridescent by oblique illumination.

In gelatine the young colonies are like particles of broken glass, clear and transparent, frequently nucleated, finely granular, and faintly iridescent. Surface expansions are whitish and even, with sharp borders. No liquefaction. There is no surface growth in bouillon; litmus milk remains blue and does not coagulate. In Dunham's solution, after forty-eight hours, there is no appearance of color after the addition of nitrite and acid. In lactose bouillon, 2 per cent, after forty-eight hours, no gas formation; glucose bouillon, 2 per cent, after forty-eight hours, no gas formation. The growth is generally diffused throughout the media, reaction neutral.

On potato there is an indifferent growth, which has slight brownish tint. At first this organism was suspected of being b. icteroides; hence its failure to ferment glucose was in accord with the reaction of the latter in this medium subjected to too great a heat in its preparation. Later this was corrected, and the organism found to have a neutral reaction in glucose and lactose media. Some nine months later, or in December of the same year, the organism introduced into animals in full doses, 2 to 10 c. c. bouillon culture, gave rise to no reaction. It was considered a pseudo-typhoid bacillus.

The most frequently isolated form of coli communis was one having the physiologic and cultural characteristics of the "X" bacillus of Sternberg and that of Havelberg, as case No. 4 will show.

Necropsy at Mercedes Hospital on a body dead of enteritis chronica.—Body of adult male; emaciation and rigidity; stomach contained a small quantity of viscid mucus, which was planted on agar. From the organs and blood developed mixed cultures; from the stomach a *pure*

one of the following organism: A rounded rod; actively motile; 1 to 3 mm. long by 0.5 mm.; pleomorphic; does not stain after Gram.

On agar plates the young colonies are opaque and whitish, becoming on the surface an amber-colored, flat, glistening expansion, with markings in the peripheral zone, and irregular iridescent edges.

On gelatine there is no liquefaction, the young colonies showing a yellow, rather opaque center, and lighter granular periphery; on the surface becoming flat, whitish, and irregular; by transmitted light more or less amber or yellowish in color and iridescent.

In bouillon a dense diffusion and some pellicle. In litmus milk it coagulates from third to sixth day with acid reaction; clot remains solid. In Dunham's solution there is much indol; in glucose bouillon, 2 per cent, forty-eight hours, one-third tube gas, $\frac{H}{2}\ \frac{CO_2}{1}$; in lactose bouillon, 2 per cent, forty-eight hours, one-fourth tube gas, $\frac{H}{2}\ \frac{CO_2}{1}$; in saccharose, 2 per cent, forty-eight hours, one-third tube gas, $\frac{H}{2}\ \frac{CO_2}{1}$.

On potato a brown scum, with much indol odor. One remarkable fact about this organism was its *perfect motility when first isolated*, which soon was entirely lost, and only a sluggish turning of the individuals upon their axes indicated *true* motion. Nor could this motility be restored, either by growth in copious fluid medium, for we used it freely in exhausting from meat bouillon the muscle sugar, and tested its motility, or from its passage through animals to which it is intensely pathogenic.

This observation corresponds with Sternberg's assertion that the bac. "X," was at first actively motile, but it is now only a sluggish roller or twister, without the least translation. We have never found this actively translatory colon in the small or large intestine—here they are all sluggish—but have found it very frequently in the blood and organs and at times in the urine and sputum.

In a number of these cases of chronic enteritis we found only a pure invasion of proteus, usually viridens, and usually accompanied by b. pyocyaneus, in some part allowing it very free albuminoid growth, such as the surface of the mesentery, from which it will grow *at first* a pure violet, but on transplantation to another tube of agar will revert to green coloration. In a case of abscess of the liver with "blue pus" the organism on agar gave bac. viridens. In but one case was there a coccus infection which evidently preceded death, and in this, diagnosed by the attending physician as "typhoid fever," we found the body emaciated, the small intestine *normal*, liver enlarged, spleen enlarged and very soft; all else macroscopically normal.

Portion of spleen incubated twelve hours and sown on agar and in bouillon, from which there came a pure culture of a group coccus, motile, not liquefying gelatine, but maintaining its coccus form. At first it was thought to be b. melitensis, but finally it lacked the rod appearance of that organism.

These bodies were not selected, except that preference was given to continued types of fever and to chronic or acute bowel diseases.

The feces were examined in 10 cases, the urine in 8. The blood in every case was freely planted from the right side of the heart; the spleen and liver invariably.

As above stated, pseudo-typhoid bacillus was isolated once, and once also from the body of one dead of yellow fever. We can but

conclude that the b. icteroides is not an ordinary denizen of the alimentary tract, whence it assists in the "secondary invasion" during an attack of yellow fever, or it would have been encountered among these diversified cases, taken from a people living in an endemic focus, and during the continuous presence of the developed disease.

One necropsy, No. 8, is of some importance because of the opinion expressed above concerning the probable mode of invasion of the b. icteroides and the seat of its localization. An adult male at morgue, unknown, save that he had staggered into a café at 10 a. m. of February 22, 1899, and died in an hour; evidently an American about 30 years of age; black hair; bloody froth from nostrils; some rigor (on 23d); much hypostasis, the skin of neck and face being bluish black; skin yellow-white; eyes yellow, pupils dilated; on section the vessels of the mesentery are engorged; the heart is in diastole, right full of very fluid blood; vasa vasorum dilated; pericardium and fluid normal, or yellowish; pleuræ normal; the lungs deeply engorged and some catarrhal pneumonia; mesentery shows numerous small extravasations between its layers; liver quite yellow and on section firm but not fibroid; it is decidedly fatty and mottled red; gall bladder contains some bile; urine is scant (3 c. c.) and albuminous; kidneys congested, but show no inflammatory changes of chronic nature; spleen is enlarged and fibrous; stomach mucosa is swollen, and eroded from post-mortem change; there are numerous extravasations into the submucosa which is stained a port-wine color; there are a few small free hemorrhages on the surface at the pylorus; the duodenum is full of dark fluid blood, its entire mucosa being extravasated and stained, that of the small intestine to a less degree; vessels of the brain and cords show congestion, the ventricles being full of serum. Cultures from heart blood and liver and the spleen and contents of the stomach.

From the findings we suggested to the medical attendant of the morgue that the case was one of yellow fever. But the blood in four tubes remained permanently sterile; the liver tubes (3) gave one growth of a motionless colon; the spleen was sterile; the blood in stomach gave a *mélange* of colon and proteus.

In this case the *odor* was intensely characteristic, as was that of the necropsy of Captain Williams, to which it bears a close comparison; and it was much more typical in all respects, save *color*, than that of Patrick Smith, undoubtedly dead from yellow fever.

Our original notes are closed with the expression, "A very promising looking necropsy gives nothing."

We now think that this case, like that of Captain Williams, was of the *siderante* type, with *no secondary invasion*. It also closely resembled that of Quintela Macias at necropsy, in which the bac. icteroides was obtained from the lung. This leads us to remark that the abuse of alcohol has long been looked upon as conducive both to contracting the disease and to its fatality, and we would suggest here the analogy between this fact and that of the frequence and fatality of pneumonia in those who habitually drink. We have no doubt that a liver almost incapacitated from fibroid changes would contribute largely toward a fatal termination in yellow fever, which so quickly disorganizes its parenchyma, but a lung deprived of its elasticity, passively congested, a ready nidus for germ infection, must play an important part not only in the contraction of the disease, but in its fatality also.

From these control cases we are prepared to say that the presence of the bacillus icteroides in any body is indicative of an infection of that body from yellow fever, although this does not question the possibility of the simultaneous growth of other pathogenic organisms and the

production of symptoms peculiar to them in such body. We have frequently noted the symbiosis of b. icteroides with the plasmodium malariæ, with the bac. tuberculosis, and in one instance with the bac. typhosus.[1]

We therefore assume that our observations fully show that the bacillus icteroides is alone associated constantly with yellow fever, is never isolated from one not infected with yellow fever, and that its presence is alone sufficient to establish the diagnosis of the disease. In this connection we ask to submit specimen tube cultures of this organism from each of our cases, each tube labeled to show its origin. It is unfortunate that through mischance of expressage the larger number of the specimens isolated from our New Orleans cultures, and from cases in Havana prior to the war, were broken and lost to us. However, the two specimens from the New Orleans work, and one from the earlier Havana work, are quite sufficient to demonstrate the validity of the former, and the identity of the organism isolated in our Southern States with that of Cuba, and the one sent us from Brazil.

REACTION OF THE BAC. ICTEROIDES TO THE PROCESSES OF DISINFECTION.

Experiment and observation having demonstrated the almost constant occurrence of the bacillus icteroides in yellow fever; its absence in ordinary diseases prevailing in an epidemic or endemic habitat of yellow fever; its pathogenicity, toxicity, and communicability, it becomes necessary now to consider its behavior to physical and chemical agents employed in sanitary work for the suppression of the disease and the prevention of its spread.

First in order comes the determination of the

Thermal death point.

[× signifies growth; — siguifies no growth. All experiments controlled.]

°C.	Minutes exposure.									
	1	2	3	4	5	6	7	8	10	12
56°	×	×	×	×	×	×	×	×	×	×
58°	×	×	—	—	—	—	—	—	—	—
60°	—	—	—	—	—	—	—	—	—	—

Result, 58° C., three minutes exposure.

The above result shows at a glance that the resistance of the organism to heat is very slight, and that a temperature far below that of boiling water would be efficient in theory, but is not, however, to be recommended in practice.

Disinfection.

CARBOLIC ACID.

Dilution.	Minutes exposure.										
	1	2	3	5	6	8	10	12	15	30	60
1-20	—	—	—	—	—	—	—	—	—	.....	.....
1-40	—	—	—	—	—	—	—	—	—	.....	.....
1-100	.....	.....	×	×	.....	.....	×	.....	—	—	—

Result, 1-20, one minute; 1-100, fifteen minutes.

[1] Sternberg quoting Agramonte. Centralblatt für Bakteriologie, Parasitenkunde und Infectionskrankheiten, Vol. XXV, Nos. 18 and 19.

Disinfection—Continued.

MERCURIC CHLORIDE.

Dilution.	Minutes exposure.								
	1	2	3	5	6	8	10	12	15
1-5,000	—	—	—	—	—	—	—	—	—
1-10,000	—	—	—	—	—	—	—	—	—

Formaldehyde gas, 3 per cent atmosphere, twelve hours exposure.—Silk thread, dry, no growth; flannel, dry, no growth; silk thread, moist, no growth; flannel, moist, no growth.

Sulphur dioxid, 4 per cent atmosphere, twelve hours' exposure.—Silk thread, recently dried, no growth; flannel, recently dried, no growth; silk thread, moist, no growth; flannel, moist, no growth.

INFLUENCE OF DRYING.

Silk threads and small pieces of flannel were thoroughly wet with a virulent bouillon culture of the bacillus icteroides, placed for twenty-four hours in the incubator to insure a good growth, and were then dried over sulphuric acid, and when thoroughly dry the following experiments were instituted, the fabrics both remaining constantly in the drying chamber until withdrawn for experiment:

Threads.—One day, growth; two days, growth; three days, growth; four days, growth; five days, growth; six days, growth; seven days, growth; eight days, growth; nine days, growth; ten days, growth; eleven days, feeble growth; twelve days, no growth.

With the flannel the results were as follows, the experiments commencing on the day when desiccation was complete:

One day, growth; two days, growth; three days, growth; four days, growth; five days, growth; six days, growth; seven days, growth; eight days, growth; nine days, no growth.

In other words, textiles of two varieties resisted a "forced" drying much more active than the natural processes for nine and twelve days, respectively.

This possibly serves to explain why the contagium of yellow fever has often proved so resistant when no disinfecting processes have been carried out, and the experiments previously detailed serve to show what comparatively feeble disinfecting agents may serve to render the contagium harmless.

The following experiment serves, too, to show what an efficient means of disinfection is furnished by nature in *sunlight*.

A piece of sterilized flannel, containing about 100 square centimeters, was saturated with a virulent, active bouillon culture of the bacillus icteroides, which flannel was then kept in the incubator for two days at 37° to insure a full and active growth. The fabric was then transferred, still quite wet, to a covered dish of clear glass, and was exposed during twenty-four hours on the roof of the building, receiving perhaps nine or ten hours of full sunlight. Pieces were then cut off and planted in tubes of bouillon. One planting was sufficient to demonstrate that the exposure as detailed above had rendered the fabric sterile; there was no growth, though the experiment was repeated and controlled.

In the climate of Havana it was impracticable to make any experiments on the action of cold, but Novy, of Ann Arbor, Mich., has had

exceptional opportunities for observation in this direction, and his experiments and observations are here quoted in extenso:

The Sanarelli bacillus will not grow at zero C. Tubes of bouillon, agar, and gelatin inoculated with this organism and kept immersed in melting ice for two weeks showed no signs of growth. At the end of that time, when placed in the incubator they promptly developed; and these cultures were as pathogenic to guinea pigs as normal cultures. Even when kept for a month at zero degree the resultant cultures were fatal as usual to guinea pigs. The minimum temperature at which the b. icteroides will grow is about 10° C. Agar and bouillon cultures were allowed to develop at this temperature in an ice chest and were then allowed to remain there for five months. In other words, they were kept for five months at a temperature of 10° to 15° C. At the end of that time these cultures were transplanted to bouillon. One c. c. of this new culture, developed for thirty-six hours at 39° C., injected intraperitoneally into a large guinea pig, produced death in six days. Evidently no attenuation had taken place, and yet under these conditions one would expect to meet with a weakened yellow-fever germ. On November 29, cultures of the Sanarelli bacillus were placed outside the window and allowed to remain there for fifteen days. The average minimum temperature for that period was 26.4° F. (—3.6° C.), and the average maximum temperature was 36° F. (2.2° C.). At the end of this time the germs grew as well as before and their virulence was not diminished. The following experiment is of still more importance. Agar cultures of the b. icteroides were placed outside the window for three days during very cold weather. During the first day the temperature ranged from —2° C. to —13° C. The temperature of the second day ranged from —10° to 20° C., and on the third day from 0° to —15° C. It should be said that most of the time during these three days the temperature was below —10° C. At the end of the first, second, and third days transplantations into bouillon were made. These bouillon cultures were developed for thirty-six to forty hours at 39° C., and the resultant growths were normal in every respect. Each culture injected intraperitoneally into new guinea pigs in doses of 1 c. c. produced death in from eighteen to twenty hours.

These experiments demonstrate (1) that contrary to the experience with cholera, drying alone is not efficiently germicidal; (2) that almost any chemical germicidal agent is efficient if its application is thorough and intelligent; (3) that, failing these, the influence of strong direct sunlight and aeration can be relied on with reasonable certainty, but that (4) contrary to the generally received impression cold alone is unreliable and inefficient (Novy).

Experiment, then, has led us to draw the following deduction: That Wasdin's experiments have shown that the avenue of infection is through the respiratory tract; that during the first stages of the disease the b. icteroides is contained alive, active and virulent, in the bronchial secretion. More attention must be paid in the future to the sputum and the dust of apartments occupied by those sick with the disease; for his demonstrations, taken in connection with our findings as to the results of drying, prove from a laboratory standpoint, what has often been suspected from an epidemiological one, that the disease spreads more in dry, dusty weather, and that the spread is temporarily diminished by showers of rain, which for a time prevent the dust of an infected city or locality being blown about.

THE PRODUCTION OF TOXINS AND ANTITOXINS.

The consideration of the chemical products elaborated by and during the growth of micro-organisms opens up a most interesting and important field of observation. These products are in general of the albumose type; they are soluble in water, insoluble in alcohol and ether, and are precipitated from solution by alcohol, and also by the sulphates of ammonium and of magnesium. They are also capable of entering into chemical combination with the salts of the heavy metals, notably gold and platinum, and thus forming double salts of very definite composition. These products being chemical in their nature are, of

course, incapable of reproducing themselves. They are of rather unstable nature; are rapidly deteriorated by oxidation, especially if in solution; are extremely susceptible to temperatures of over 60°, and are rendered largely inert by any prolonged or active chemical manipulation. Their ultimate chemical composition has not as yet been fully studied, but it can be conjectured from their action on the animal economy that they are alkaloidal in nature.

In the widest and most general sense of the term there are two forms of the substances, or possibly two distinct substances, probably the former. One is liberated during the process of bacterial growth, preferably in liquid media, small in quantity and of feeble intensity; the other is enveloped and contained in the microbial body; is liberated when the ectosarc is dissolved by an appropriate reagent, is larger in quantity and far more intense in action. A bouillon culture of a pathogenic microorganism of appropriate age, the microbial growth being arrested by heat, by chemical means, or by filtration through porcelain, constitutes one form of toxin, very potent in the case of some bacteria, as in the diphtheria bacillus, but very weak in the case of the b. icteroides and some others, notably the b. pestis. Three methods of the preparation of toxins from the b. icteroides will be here described.

(1) This form will be spoken of simply as "toxin," and was prepared by treating a fifteen-day old bouillon culture of the b. icteroides, ripened in a Fernbach flask at 37°, with a few drops of chloroform or by heating the culture at 60° for one to two hours.

(2) A much more potent toxin can be prepared from the b. icteroides by treating large quantities of culture grown on agar-agar by a solution which dissolves the ectosarc or outer layer of the bacterial cell, and liberates the enveloped toxin. This was prepared by inoculating agar on a large "moist chamber" (a Roux culture flask is preferable) by means of a swab, with a virulent bouillon culture of the b. icteroides. Grown at 37° for forty-eight hours there results a very heavy growth, which is scraped off the surface of the medium with a sterilized spatula, cover slip, or other convenient appliance. These scrapings are put into a small flask, the denuded surface of the medium washed with a little sterilized distilled water to remove as much of the growth as possible, and to the scrapings and washings in the flask is added a quantity of 0.6 per cent solution of potassium hydrate, and the flask securely stoppered. In a few days a remarkable change takes place in the contents of the flask. The microbial mass swells enormously, the liquid becomes thick and grumous, and then gradually the mass contracts and a clear yellow liquid is exuded. Culture from the flask from time to time shows that microbial life has been arrested, and microscopic examination of the deposit at the bottom of the flask shows bacterial detritus, but no complete forms. The body of the microbe has been dissolved and its enveloped toxin set free. Toxin thus set free will, in this report, be denominated "soluble," and is of special value in the immunization of animals in the preparation of antitoxic serums.

(3) Still another form of toxin was prepared and experimented with. Bouillon, containing in addition to its ordinary constituents 0.5 per cent gelatin, was put into a Fernbach flask, inoculated heavily with a virulent bouillon culture of the b. icteroides and allowed to ripen at 37° for fifteen to twenty-one days. The culture was then killed by a few drops of chloroform, the liquid put into a large beaker, thoroughly saturated with ammonium or magnesium sulphate, and a large excess of the precipitating substance added in crystals.

The toxin is precipitated out of solution in floceuli, which are ren-

dered larger by the gelatin added to the bouillon, and owing to the high specific gravity of the liquid by the addition of the salts the flocculent, curdy mass rises to the top of the liquid. This is collected by straining through linen, dissolved in the least possible quantity of distilled water, and the solution filtered into strong or preferably absolute alcohol. A grayish-white amorphous precipitate results, which is collected on a filter, and can again be dissolved in water and reprecipitated by alcohol. Too frequent solution and precipitation renders the substance difficultly soluble in water, but increases its purity. It is finally collected on a filter, washed twice each with absolute alcohol and ether, dried in the air rapidly, then in vacuo over sulphuric acid, and reduced to powder in a sterilized mortar. The resulting substance will be designated "precipitated toxin." In our experiments, it was elected to precipitate by magnesia rather than by ammonia salts, for the reason that mice were the animals selected for the experiments, and the injection of considerable quantities of ammonia compounds into small animals is a process not unattended by danger.

With these substances Nos. 1, 2, and 3, the following experiments were performed. Toxins were prepared by method 1 from b. icteroides, b. typhosus, bacillus "X" (Sternberg), and b. coli communis.

February 14, 1899.—White mouse received subcutaneously 0.25 c. c. toxin b. icteroides; found dead at 8.15 a. m., February 15, 1899.

February 14, 1899.—White mouse received subcutaneously 0.25 c. c. toxin b. typhosus; sickened, but recovered.

February 14, 1899.—White mouse received subcutaneously 0.25 c. c. toxin bacillus "X" (Sternberg); no effect.

February 14, 1899.—White mouse received subcutaneously 0.25 c. c. toxin b. coli communis; no appreciable effect.

Of the toxins prepared by this method that of the b. icteroides alone was sufficiently potent to kill a white mouse weighing 17 grams.

Another series of experiments was as follows, toxins method No. 2:

February 25, 2 p. m.—Injected large white mouse with 0.10 c. c. soluble toxin b. icteroides. Found dead 8.30 a. m., February 26, 1899.

February 28, 1.30 p. m.—Injected large mouse with 0.20 c. c. soluble toxin b. icteroides. Found dead at 8.30 a. m., March 1, 1899.

February 28, 1.30 p. m.—Injected very large mouse (33 grams) with 0.15 c. c. soluble toxin b. icteroides. Found dead at 8.30 a. m., March 2.

February 28, 1.30 p. m.—Injected large white mouse (26 grams) with 0.10 c. c. soluble toxin b. icteroides. Found dead at 8.30 a. m., March 1.

As controls to the above experiments two mice were injected with respectively 0.10 and 0.20 c. c. sterilized 0.6 per cent solution of potassium hydrate. No ill effects resulted.

These experiments demonstrated that the toxin produced by the b. icteroides in ordinary process of growth is of somewhat feeble intensity, (2) but that there is contained within the microbial body of the same organism a toxin of considerable potency, easily released by an appropriate reagent.

With the precipitated toxins (method 3) of b. icteroides the following experiments were performed:

February 27.—Mouse (weight 25 grams) injected subcutaneously with 0.1 gram precipitated toxin b. icteroides; dead in twenty minutes.

February 27.—Mouse (weight 23 grams) injected subcutaneously with 0.1 gram precipitated toxin b. icteroides; dead in fifty-two minutes.

February 27.—Mouse injected subcutaneously with 0.09 gram precipitated toxin b. icteroides; dead in twenty-eight minutes.

February 27.—Mouse injected subcutaneously with 0.07 gram precipitated toxin b. icteroides; dead in one hour and twenty minutes.

February 28.—Mouse injected subcutaneously with 0.05 gram precipitated toxin b. icteroides; found dead 8.30 a. m., March 1.

February 28.—Mouse injected subcutaneously with 0.03 gram precipitated toxin b. icteroides; found dead after several days.

February 28.—Mouse injected subcutaneously with 0.01 gram precipitated toxin b. icteroides; rendered very sick, but survived.

As it was known that the toxin as prepared contained a considerable quantity of the precipitating agent—magnesia in this instance—a quantitative estimate was made of this substance by precipitating as ammonio-magnesian phosphate and subsequently burning to magnesian pyrophosphate. This gave MgO 79.98 per cent; consequently the dose in all the above experiments of actual toxins, or rather of mixed albumoses, is represented by 100—79.98=20.02 per cent—practically 20 per cent of the dosage set forth.

These experiments satisfactorily demonstrated to us the very intense toxicity of the substances isolated and the highly virulent nature of the organism of which we were taking account. We noted that the effects of the toxins were almost fulminant in character; that often before the mouse was released from the forceps which held him he was practically in articulo mortis, profoundly unconscious to external stimulations, the legs spread out, and the animal as if flattened by some heavy weight; respiration very much hurried and shallow, gradually becoming gasping and diaphragmatic; the action of the heart becoming more and more labored. The effects of the substances seemed to be exerted on the whole axial nervous system, especially on the respiratory and circulatory centers. In the cases where recovery took place after the smaller doses, it was noted that the effects of the intoxication seemed very transitory; that the animal recovered as if from a profound narcosis, and while quite sick for several days gradually recovered tone and vigor. How truly this corresponds to the clinical picture in the human being suffering from yellow fever, where the individual at the very verge of dissolution is suddenly restored to a species of semi-existence by the resumption of the function of the kidneys, and gradually goes on to a delayed but full convalescence.

With a view to determining the relative as well as the actual toxigenic power of the b. icteroides, toxins were prepared from the b. coli communis and the b. typhosus. The former was injected into a mouse in doses of 0.1 gram without fatal results; the latter was tried repeatedly until it was established that, while an animal of average size would support doses of 0.5 and 0.6 gram without ill effect, a dose of 0.7 gram killed in one hour and forty minutes. Making due allowance for the 80 per cent magnesia contained in the toxins precipitated by this method, it still makes the lethal dose of the typhoid toxin prepared by us many times larger than that of the typhotoxin prepared by Vaughan and Novy, whose method, however, it was not convenient to pursue.

Efforts were not wanting on our part to eliminate the large content of magnesia and to obtain our toxic substances in a state of greater purity and concentration. It was a simple matter to eliminate all the magnesia by precipitation as ammonio-magnesian phosphate, separating this by filtration and precipitating the filtrate with absolute alcohol. This precipitate was collected and dried, was found to be with difficulty soluble in water, and had lost its toxic power; a mouse sustained a maximum dose (0.1 gram) without any ill result.

While much work has been done abroad and by Vaughan and Novy in the United States in the isolation of the toxic substances of the

pathogenic bacteria, it is obvious that more finished means to this end is a desideratum. The methods pursued by us are, practically, those pursued at the Institut Pasteur in the preparation of toxins as one step in the immunization of horses against the b. pestis.

An impression has long prevailed that the elimination of the toxins of the yellow-fever organism occurred through the kidneys; indeed efforts have been made to prepare an antiamaryllic serum by the immunization of horses by means of the urine of yellow-fever patients and convalescents. In our experiments it was noted that those mice which urinated freely during the continuance of the intoxication seemed to survive longer or even to recover. Our assistant, Acting Assistant Surgeon Menocal, was therefore directed to try the precipitation of toxic substances from the urine of three cases of yellow fever (one ending in death and two in recovery) by two methods: (1) by precipitation along with the serum albumen by means of absolute alcohol, and subsequent purification by dialysis; (2) by precipitation along with the albumoses and globulins, with subsequent purification by solution in water and reprecipitation by alcohol, essentially as described in method No. 3.

The following experiments were made:

With toxic substances precipitated from the urine by alcohol, from a severe, nonfatal case of yellow fever: Mouse injected subcutaneously with 0.06 gram; dead in one hour and thirty minutes.

With toxic substances precipitated from urine of fatal case of yellow fever (precipitated by alcohol): Mouse injected with 0.06 gram; dead in fifteen minutes.

With substances precipitated by alcohol from the urine of a mild case of yellow fever: Mouse injected subcutaneously with 0.06 gram; rendered very sick, but recovered.

With toxic substances precipitated by means of magnesian sulphate (globulins and albumoses) from urine of fatal case: Mouse injected subcutaneously with 0.05 gram; dead in three hours.

With substances precipitated by sulphate magnesia (globulins and albumoses) from urine of mild case: Mouse injected subcutaneously with 0.05 gram; sick, but recovered in a few days.

These experiments are interesting as seeming to prove the contention given above as to the elimination of the toxins, and also as seeming to show that in a fatal, a grave, and a mild case the poteney of the substances and the time of death of the animals subjected to experiment was in direct relation to the gravity of the case.

An impression has prevailed, too, and has been advanced as a fact, even, that be the specific cause of yellow fever what it might, there was a large element of danger in a secondary infeetion by means of the b. coli communis. If this be so it would seem naturally to be due to an absorption of elaborated toxins; and while the theory is admitted as a possibility the following experiment would seem to negative the idea:

A mouse injeeted subcutaneously with 0.05 c. c. soluble toxin b. icteroides dead on eighth day.

A mouse injeeted subcutaneously with 0.05 c. c. soluble toxin b. icteroides +0.02 gram precipitated toxin b. coli commun., survived without any ill effect.

It is here permissible to refer to a theory advanced by one of us (Geddings) in 1894, that a part of the difficulty of isolating a specific microbe in yellow fever was due to the possibility that the specific organism was inhibited or destroyed in the substances elaborated in

its process of growth (toxins). The following experiment made by us would seem, in a measure, to confirm this theory:

Into a test tube containing 10 c. c. of bouillon was put 0.20 c. c. of soluble toxins b. icteroides, and in this mixture was planted an active culture of b. icteroides. In twenty-four hours there was an evident precipitation at the bottom of the tube, but no growth was obtained by inoculating other bouillon tubes. The inoculations were repeated from tube to tube for six generations without result of any growth. The experiment was repeated by using a quantity of the precipitated toxins of the b. icteroides 1–100. Similar results were obtained—a precipitation in the first tube and no growth in a series of tubes planted from it.

A still further test was made: Three tubes containing precipitated toxin 1–100, as above, were inoculated, respectively, with b. icteroides, bacillus "X" (Sternberg) and b. coli communis. In the first tube containing the b. icteroides there was a precipitation, but it was impossible to obtain a growth by transplantation. The bacillus "X" and b. coli communis seemed very slightly inhibited in the first tube, but from subsequent transplantations gave cultures normal in every respect.

On the contrary, the toxin of the b. coli communis has no effect upon the growth of the b. icteroides, as is demonstrated by the following experiments: To a tube of bouillon was added precipitated toxin of the b. coli communis in the proportion of 1–100, and in this tube was planted a culture of the b. icteroides. In twenty-four hours the tube presented the appearance of a culture of the b. icteroides normal in every respect, and transplantations to other tubes of bouillon and agar-agar confirmed this view. Therefore the toxin of b. coli communis is not inhibitory or germicidal to the b. icteroides.

But little can be said here as to the use of the "serum antiamaryll" of Sanarelli, either for or against. As directed by the Surgeon-General of the Marine-Hospital Service, an effort was made in 1897–98 to introduce its use into the Spanish military hospitals in Havana, and while, as usual, the request was politely entertained, from the questions asked and the nature of the information requested it was obvious that full permission would never be accorded. Surgeon Wasdin had a limited opportunity to observe its effects in New Orleans in 1898, and felt obliged to report that the case in which there was a favorable termination after its use would have probably recovered without it, and that it was without effect in a grave case, finally terminating fatally.

The action of serums may be broadly divided into three classes, (1) those which prevent infection by their influence on the economy, stimulating the leucocytes to increased activity against the living germ (phagocytosis); (2) those which, in addition to the above property, stimulate the *whole* cell economy to the production of an antitoxin, or supply in themselves an antitoxin for the neutralization of the toxins produced by growing and multiplying microorganisms; (3) those whose power is exercised directly upon the microorganism itself, having no power to stimulate phagocytosis, or to supply or cause the elaboration of an antitoxin—the so-called "alexins." To this class it would seem that the "serum antiamaryll" must be relegated.

The following experiment was made:

Mouse inoculated at root of tail with 1 loop of seven-day-old culture b. icteroides, dead on seventh day.

Mouse inoculated at root of tail with 1 loop of seven-day-old culture b. icteroides with 0.20 c. c. serum antiamaryll dead on tenth day.

The animals being of practically equal size and weight, and the quantity of culture administered being equal, the most that can here be claimed is that the serum prolonged life for three days, but in no way prevented a fatal issue.

A brief exposition may here be entered into as to the requirements of an efficient serum or antitoxin. Experience has demonstrated that in the case of several of the pathogenic organisms it was a comparatively simple matter to immunize a horse or other domestic animal to a point where a dose of the said serum, usually large, would either prevent infection or modify it to a considerable extent. Such serums *may be* simply *alexic*, or may, as before said, act by stimulating the leucocytes to increased phagocytic action. They can be only considered, therefore, as *protective* or *preventive* serums. To claim power as a true "antitoxin" much more is required. In this case not only must the agent protect against culture (living organisms), but it must go much further, and be proved to be capable of neutralizing the toxins of a disease after infection has fully taken place and the disease in question is well established. As an instance: It was comparatively an easy matter in the preparation of the antipest serum at the Institut Pasteur to prepare a serum which would protect mice, rats, and guinea pigs against a mortal dose of a culture of the b. pestis, but a far different matter to prepare one which would protect similar animals against toxins of the disease, isolated and administered, or which would prevent a fatal issue when the malady was once established by inoculation with the organism. An antipest serum, therefore, was not pronounced effective until it had not only proved its efficacy against a mortal dose of the live organism, but *until a dose of 0.10 to 0.20 c. c. would protect a mouse against a four-times mortal dose of the precipitated toxins.* This requirement may seem exalted, but is, in our opinion, just and proper, and we believe that until a serum against yellow fever somewhat approaching these requirements shall be produced, the question must be regarded as still unsettled.

Therefore, with no anticipation that the serum antiamaryll of Sanarelli would answer the requirements, or indeed, that at this stage it would be fair to demand that it should, the following experiments were instituted:

February 26.—Mouse received 0.10 c. c. soluble toxins b. icteroides at 2 p. m.; found dead at 8.30 a. m., February 27.

February 26.—Mouse received 0.10 c. c. soluble toxin b. icteroides, with 0.20 c. c. serum antiamaryll, at 2 p. m.; found dead at 8.30 a. m., February 27.

February 27.—Mouse received 0.1 gram precipitated toxin b. icteroides; dead in twenty minutes.

February 27.—Mouse received 0.1 gram precipitated toxin b. icteroides with 0.20 c. c. serum antiamaryll; dead in forty-five minutes.

Therefore it must be admitted that the serum failed signally to protect an animal against toxin, and consequently that it can not be claimed to be a true antitoxic serum or antitoxin.

EXPERIMENTS ON AGGLUTINATION AND ARREST OF MOTILITY.

The wide application of the reaction of Widal, its modification at the hands of Wyatt Johnson in the United States, and the published work of Archinard and Woodson of the results of their experiments in New Orleans during the epidemic of yellow fever in 1897, rendered it necessary that we should make every effort to supply some test sim-

ilar to the Widal as a means of rapid and ready diagnosis of yellow fever. Experiments were therefore made on b. icteroides with various serums and in varying dilutions; with the blood of yellow fever patients and with the blood of animals sick or dead as the result of inoculation with b. icteroides. The results were most varying and bewildering, and convince us that whatever may be the value of the reaction as a diagnostic point in enteric fever, it has little or none in yellow fever. Following is a transcript of the experiments made.

B. icteroides with serum antiamaryll, diluted 1–4,000.

	Fifteen minutes.	Thirty minutes.	Sixty minutes.
Agglutination	A small clump	No additional clumping.	Decided clumping.
Arrest of motility	Unchanged	Slightly impaired	Decided impairment.

B. icteroides with Serum Antiamaryll, diluted 1–2,000.

Agglutination	Slight	Increasing	Decided.
Arrest of motility	Impaired	Complete arrest	Absolute arrest.

B. icteroides with Serum Antiamaryll, diluted 1–1,000.

Agglutination	None	None	Practically none.
Arrest of motility	Not impaired	Not impaired	Not impaired.

Claims having been made that the motility of the B. icteroides was affected and agglutination produced by various other serums (antitoxins) and by the serum of normal human blood, the following experiments were made, the dilution being 1–10 unless otherwise stated, and the times of observation fifteen, thirty, and sixty minutes, respectively:

B. icteroides with New York Health Department diphtheria antitoxin 1–10.

	Fifteen minutes.	Thirty minutes.	Sixty minutes.
Agglutination	None	None	Practically none.
Motility	Not impaired	Not impaired	Not impaired.

B. icteroides with antipest serum (Pasteur Institute) 1–10.

Motility	Slightly diminished.	Slightly diminished	Slightly diminished.
Agglutination	None	None	None.

B. icteroides with serum antivenin (Pasteur Institute, Lille) 1–10.

Agglutination	None	None	None.
Motility	Unchanged	Unchanged	Unchanged.

B. icteroides with typhoid serum[1] *1–40.*

Agglutination	Slight	Slight	Slight, but marked.
Motility	Impaired	Impaired	Much impaired.

[1] The typhoid serum here referred to is the serum of a horse treated with killed cultures of the b. typhosus, and having an agglutinative reaction on the b. typhosus in the proportion of 1–200000.

B. ieteroides with typhoid serum 1–100.

Agglutination	None	None	None.
Motility	Impaired	Impaired	Impaired.

B. ieteroides with heart blood of rabbit injected with organism from control necropsy No. 6.—After an exposure of two hours and forty-five minutes there is a slight formation of small clumps, but no general agglutination and no impairment of the motility of individual organisms.

Blood of P. W. S. (a case of yellow fever), dilute 1–10.—After an exposure of two hours and fifteen minutes there is no agglutination and no arrest of motility.

The same diluted 1–20.—After an exposure of one hour there is no agglutination and no arrest of motility, and the experiment was suspended on account of the drying of the drop.

B. ieteroides treated with the blood of Atwood, diluted 1–20.—Fifteen minutes, diminished motility; thirty minutes, much diminished motility; formation of decided clumps; forty-five minutes, motility progressively diminishing, and size of clumps increasing; sixty minutes, motility in general diminished at least four-fifths; impaired motility of all organisms, and total arrest of the large majority. General formation of large clumps.

This result possessed much interest, being arrived at in the first few days of Mr. Atwood's illness. It became much more so when subsequent developments proved that the case was one of *gastric ulcer* and not yellow fever.

Appendix A.

NOTE ON THE SIMILARITY BETWEEN BAC. ICTEROIDES AND BAC. CHOLERÆ SUIS.

Almost at the close of our work in Havana there appeared in the Medical News of April 29, 1899, a communication from Drs. Reed and Carroll, of the Army laboratory, in which they called attention to a marked resemblance between these two organisms—a resemblance culturally so striking as to induce these observers to submit the domestic hog, a special reagent of bac. choleræ suis, to the influence of bac. icteroides placed in their food. This resulted in the infection of a number of these animals in which necropsic examination showed the typical ulcerations of the small intestine found in those dead of choleræ suis.

Only two cases of this infection are given in the communication mentioned, in reality but one, since the second pig merely died of what the first one did, having been fed upon its infected organs. From our experience given above, with two domestic pigs, six weeks old, fed upon the most virulent cultures of bac. icteroides, we can but doubt the accuracy of these observations, and are forced to reflect that from the fact of the observation of this resemblance the bacillus choleræ suis must have been brought into association with bac. icteroides. If so, there exists the possibility of a mixed culture of these so similar organisms, and this fed to domestic hogs would undoubtedly terminate fatally, with the characteristic ulcerations of hog cholera. These reflections arise from our contrary experience with pure cultures of the bac. icteroides, and as the result of our experiments with this bacillus upon hogs at the Delaware Breakwater Quarantine free from any probability of infection from the bac. choleræ suis.

Reverting to the communication above mentioned, we must express our surprise that these observers, after stating that the organism of Sanarelli possesses a most marked specificity, equaling that of b. choleræ suis, of which they consider it a variety, advance the proposition that it, when found in cases of yellow fever, is one of the secondary organisms, such as the colon and proteus, which invade the body in the "last hours of life."

From this we could almost assume that the organisms in question can originate de novo, and if it can give rise to yellow fever, then the old theory of the spontaneous evolution of this disease is again introduced. We can not assent to this proposition. The work of Sternberg, who says, "In the series of cases studied by me secondary infections were extremely rare,"[1] and the work of Sanarelli and our own is opposed to the barest possibility that the bac. choleræ suis could have been present in the intestinal content and not have been discovered.

As before intimated, Dr. Sternberg's work was too limited, its field too narrowed for the detection of the organism, for he confined his

[1] Annual Report Am. Pub. Health Assn., 1898.

observations principally to the alimentary tract, and in at least half of his necropsies does not mention planting the heart blood. In making this statement we realize the advances made in bacteriologic technique in the decade that is passing, nor can we underestimate the fortuitous chance that placed in Sanarelli's hands such cases as his No. 2 and No. 8, cases only approached in the case of isolating the germ by the one, in our experience, of Patrick W. Smith of our series.

On May 25 two fawn-colored mice were placed in a sterile glass case, and 2 c. c. of fresh bouillon culture of bac. choleræ suis added to their water and food.

June 4 one mouse was found dead, just ten days from time of exposure. Necropsy showed much engorgement of the vessels of the skin and mesentery; the liver was reddish in color; spleen normal or enlarged a little; kidneys congested; urine, none; right heart full; pericardium and pleuræ normal; stomach contains no black vomit; mucosa congested; that of duodenum coated with bloody mucus; lungs apparently normal; cultures from the blood all gave a pure culture of the bac. choleræ suis.

On the 5th, at 6 a. m., the second mouse is found dead. Necropsy shows decomposition of the organs; the liver is yellowish; the spleen is much enlarged; stomach normal; small intestine congested. From blood planted there develop pure growths of the bac. choleræ suis.

On May 28 two large brown rats, from the same source, were caged separately in sterile glass cages, and to one was given 2 c. c. of fresh bouillon culture of bacillus icteroides from Bologna, and to the other 2 c. c. of fresh bouillon culture of bacillus choleræ suis.

On June 1 there was again given 1 c. c. of the same organisms to the respective rats.

On the 4th the hog-cholera rat seems quite sick; the other very droopy.

On 6th both are doing very well.

On 8th both improving, and are killed with formaldehyde gas, because of closure of our laboratory; no necropsies.

Attention is called to the same result in the case of the large white rat fed on b. icteroides, whose history is given above.

THE "PFEIFER REACTION" TEST BETWEEN THE BAC. ICTEROIDES AND BAC. CHOLERÆ SUIS.

On May 19 two large fine guinea pigs were selected, as nearly alike in size and weight as possible, their abdomens shaved and sterilized and the skin incised with one stroke of the sterile scissors down to the muscular walls, then with sterile glass pipettes, readily forced through the parietes thus exposed, we introduced into the cavity of the abdomen of pig No. 30 1 c. c. of a mixture of a twenty-four-hour bouillon culture of bac. icteroides (Smith) and serum antiamaryl of Sanarelli in the exact proportion of 1–20. Also, and coinstantaneously, into pig No. 31 we introduced 1 c. c. of a similar culture of bac. choleræ suis and serum antiamaryl of Sanarelli, in the exact proportion of 1–20. Both animals stand the quick punctures well. In twenty minutes a few drops of fluid were withdrawn from the cavity of each pig and immediately examined in hanging drop. The bacilli icteroides were found *motionless*, more or less granular and deformed or bent, and tending to groups of several elements agglutinated. Stained, this cover slip showed *less reaction* to methyl blue after long exposure; a granular appearance of the bacilli; some were vacuolated and many in small

clumps. In forty minutes the same pig gave serum which in hanging drop showed fewer bac. icteroides, more granulation and distortion of the rods, many fragmented and none in motion. This cover slip stained in methyl blue gave small aggregations of disorganizing rods, granular, and not reacting well to the stain. At the end of sixty minutes we found the serum so bloody that no correct observation could be made.

In pig No. 31, at the end of twenty minutes, the extracted serum gave hanging drops in which numbers of bac. choleræ suis could be seen under strong inhibition, the motion being very slight and rotatory. The end of the rods impinging against the glass gave them the appearance of cocci. None were observed in the condition of granulation or of deformity, although there were a few aggregations of elements. Stained with methyl blue they reacted normally. In forty minutes the rods were fewer in number, but of sound appearance, and reacted well to the stain.

As in pig No. 30, I found the abdomen so full of blood at the end of sixty minutes that no observation could be made.

These microscopic observations bear closely upon the observations of Pfeifer in the case of his antiinfectious serum of cholera, and he claims that under these conditions no organism will be affected except those of the same kind. From them we would say that b. choleræ suis is absolutely distinct from b. icteroides. However, similar experiments with pigs injected with icteroides and serum antiamaryl have ended with us in the death of the pig.

On May 19 both pigs have reacted well; are quiet, and do not try to eat. They remain ill until the 26th, when No. 31 dies at 4 p. m.

On the 27th No. 30 is found dead at 6 a. m., evidently for several hours.

Necropsy No. 31.—Done at once. Skin vessels are congested, as are those of the mesentery; the liver is of a yellowish color and mottled and looks *fatty;* the spleen is *greatly* enlarged; the lungs are congested; right heart distended; the kidneys are swollen; the mucosa of stomach is normal; its content greenish; the duodenum contains some blood-stained mucus; mucosa is stained; the uterus is much congested; urine contains 3 per cent moist albumen. With osmic acid the liver cells show fatty degeneration quite extensively. Liver gives amount of extracted fat by weight: Fresh, 10.000 grams; dried, 2.625 grams; residue, 1.975 grams; fat, 0.650 gram, or 6.5 per cent of fresh and 25 per cent of dry.

Cultures were taken from heart and spleen and both gave pure cultures in abundance in twenty-four hours of a bacillus that differs from b. icteroides in producing H_2S in bouillon cultures, or choleræ suis.

Necropsy of pig No. 30.—Several hours dead; vessels of the skin distended; tissues dry; mesentery of small intestine engorged; the liver is of a light yellow tint and friable; spleen is normal in size; right heart distended; the pleuræ ecchymotic; lungs deeply congested; stomach mucosa deeply stained by submucous extravasations, but there is no free blood; the duodenum appears the same; bladder contains albuminous urine. Hepatic tissue teased in osmic-acid solution gave extensive fatty change in the cells, there being very few cells in which the black granules of fat were not seen.

Extracted fresh with ether and this weighed gave in 10.000 grams 1 gram of fat, or 10 per cent; dried, 2.670 grams, or 37.23 per cent, fat; residue, 1.670 grams; fat, 1 gram; cultures from blood; the organs fed to two domestic pigs.

In twenty-four hours one of these tubes appears sterile, on the other one there is a suspicion of growth. After forty-eight hours the latter has from 5 to 10 isolate colonies, quite small; the first has three minute isolate colonies and a better growth in the water of condensation.

In the case of pig No. 31, the tubes were beautifully grown at the end of twenty-four hours, and have continued in marked contrast to the inhibited growths from pig No. 30.

Both organisms are translatorily motile. Therefore, although the serum antiamaryll is not an antiinfectious one, and does not in its present potency result in saving the animal from infection, it has in our hands lengthened the period of disease several days in animals.

EXPERIMENTS AT DELAWARE BREAKWATER QUARANTINE.

The following is the result of the experiments mentioned above, to determine any possible relation between the b. icteroides and the b. cholerae suis:

At the quarantine station at Delaware Breakwater seven pigs of the same litter and about two months old were put into a new sty, and kept under observation for three or four days to assure ourselves of their freedom from any disease. They were then separated and six were put into one pen and one into another to serve as a control.

July 4, 1899.—To the six pigs were fed the following: 12 cultures on agar-agar of the b. icteroides, forty-eight hours old, derived from culture received direct from Professor Sanarelli at Bologna; 12 cultures on agar-agar of the b. icteroides, forty eight hours old, derived from culture isolated by us in Havana, Cuba; 500 c. c. of bouillon culture b. icteroides, forty-eight hours old, derived from Sanarelli's Bologna culture.

July 5, 1899.—Pigs appear in perfect health. Fed with 500 c. c. seventy-two-hour-old bouillon culture b. icteroides, derived from Sanarelli's Bologna culture, and on the evening of the same day 500 c. c. more of the same culture.

July 6, 1899.—The pigs seem in perfect health and during the day receive a further feeding of 500 c. c. of a similar bouillon culture of the b. icteroides.

Therefore in forty-eight hours the six pigs receive 24 agar cultures, and 1,500 c. c. of bouillon cultures of the most virulent specimens of the b. icteroides of which we are in possession.

The pigs were carefully watched during the time intervening until July 14 (ten days from the first feeding), and at no time was there any loss of appetite, or any indication of sickness whatsoever. On this day, July 14, three of the fed pigs and the control were killed. The stomach, organs, and the intestinal canal of the control pig were normal in every respect. The stomachs, organs, and intestinal canals of the three fed pigs presented no lesions indicative of any infection from the b. icteroides, *and were absolutely and entirely free from any appearance which could be considered as indicative of the well-known lesions of hog cholera.* Further, at this date, July 23, the three remaining pigs have remained in perfect health and show no indications either of the disease produced by the b. icteroides (yellow fever) or of hog cholera.

From these experiments, then, we deduce—

I. That the domestic pig is incapable of infection by the b. icteroides when introduced through the intestinal or digestive tract.

II. That the b. icteroides when fed to pigs will *not* produce any of the symptoms or intestinal lesions characteristic of hog cholera, as claimed by Reid and Carroll.

APPENDIX B.

INVESTIGATION INTO THE CAUSE OF YELLOW FEVER.

PRELIMINARY REPORT OF MEDICAL OFFICERS DETAILED BY DIRECTION OF THE PRESIDENT AS A COMMISSION TO INVESTIGATE IN HAVANA THE CAUSE OF YELLOW FEVER.[1]

SIR: I have the honor to submit the following general summary of the work of the commission appointed by you, with the consent of the President of the United States, for the purpose of investigating the etiology of yellow fever.

Introductory to this, I must mention the work done by me, prior to this detail, in this direction, during the prevalence of the yellow fever along the Gulf coast of Mississippi and Louisiana during October and November of 1897.

In compliance with your directions I had commenced such investigation at Ocean Springs, Miss., during the latter part of September, visiting a number of cases, and securing tube cultures from the living blood. Early in October, through the courteous invitation of Dr. S. R. Olliphant, president of the board of health of Louisiana, and of Prof. P. E. Archinard, bacteriologist to the board, and by your permission, I joined Dr. Archinard in the investigation being conducted by him in New Orleans, where the fever existed. This investigation had been instituted by the authorities of the Charity Hospital, and to Dr. Bloom, the courteous superintendent and resident surgeon of that institution, I am indebted for the privilege of visiting the isolation division of that hospital, and the valuable opportunity of working in conjunction with the gentlemen detailed for that especial duty. Other than a study of the large number of cases of the disease at this hospital, which I estimate an invaluable experience, I entered actively into the pathologic and bacteriologic work. A detailed description of this work would anticipate the report of the pathologist in the proposed general report of the epidemic by the authorities of the Charity Hospital, since it was assumed that any and all work done would be for the use of that institution. It suffices to state that it was impracticable to make, during the limited continuance of the fever, extensive bacteriologic examination of any individual case, owing to the number of necropsies held during this time; and it was necessary to postpone examination in most cases to a later date, the cultures from the blood and organs being carefully preserved, together with those obtained at Ocean Springs.

I should here refer to the uniform courtesy of the gentlemen who formed the general staff of the isolation hospital, Drs. Veazie, Hamilton, Jones, and Pothier, with whom it was a pleasure to be associated. The bacteriologic work was carried on in the laboratory of the medical

[1] Reprint from Public Health Reports, November 11, 1898.

department of the Tulane University, under the direction of Professor Archinard, from whom, and the dean of the college, Prof. S. E. Chaille, many courtesies were received by myself and P. A. Surg. H. D. Geddings, who had joined me in this portion of the work. Upon the receipt of your order on November 11, 1897, to proceed to Havana and there continue the investigation into the cause of yellow fever, we proceeded to do so as early as practicable, all the culture material, almost entirely obtained from necropsies, little attention having been devoted to fresh blood plantings, being carefully transferred to that city.

Our reception by the Captain-General of Cuba, General Blanco, was marked by a kindness which presaged the most satisfactory arrangements for the conduct of the investigation, and we received an early introduction into the military hospital of Alphonso XIII, that at Regla, and that of St. Ambrosio. Owing to unavoidable delays the laboratory installation was not completed until near the middle of January, 1898, and from this time until our work was discontinued, on March 16, by your direction, the number of cases of yellow fever was very limited. It was during this period that opportunity was had to thoroughly examine the cultures obtained in the United States. Here I must state that an impetus had been given the matter of etiologic investigation by the Annales de l'Institut Pasteur for June, 1897, of the claim by Prof. Guiseppe Sanarelli, of the University of Montevideo, Uruguay, that he had discovered in his bacillus icteroides the cause of this disease.

The semidetermination of Dr. G. M. Sternberg, U. S. A., that the bacillus X (bacillus cuniculicida Havaniensis), discovered by him in 1889, was the cause of this fever, had been withdrawn,[1] and the claim of other discoverers negatived,[2] so that it was incumbent upon us to at once ascertain whether the organism which Sanarelli described, the bacillus icteroides, was to be found in the cultures made by me in New Orleans, and, if so, to ascertain by comparative necropsies, performed upon bodies of those dead from disease other than yellow fever, whether this new organism had been overlooked, or not detected by Sternberg in his justly celebrated work in this field, since he had declared that there was nothing in the blood or organs of yellow fever patients which he had not detected. To this end each case necropsied at New Orleans was patiently and thoroughly plated from young bouillon cultures from the original and subsequent plantings, these originals having been taken from blood, spleen, liver, and kidneys, the portions of organs in many cases having been incubated twelve hours prior to the inoculation of the tubes, as advised both by Sternberg and Sanarelli. Besides these cultures, those taken from living patients at the isolation division of the Marine-Hospital detention camp at Fontaineblean, Miss., were also carefully examined. It was from the blood of one of these cases, "Goodrich," planted on September 28, 1897, that the most typical forms of colonization, to those described by Sanarelli for bacillus icteroides, were noticed, of a small rod, actively (at first) motile, not retaining the stain after Gram, but which later in New Orleans gave rise to the production of indol and the formation of gas with both glucose and lactose. This organism, as at first noted, more nearly resembled that of Sanarelli than any other isolated at the time we left the laboratory at New Orleans, and it was now taken up for more careful observation than had been then possible. For unanimity of purpose it was thought best that each of us should prepare, inde-

[1] Report on yellow fever; Sternberg, 1890, U. S. M. H. S.
[2] Idem.

pendently, cultures of each and every specimen on hand, and carry them to a definite termination, a decision which afforded much satisfaction at the termination of the tedious and exacting labor of plating and replating so large a number of organisms, in that while we succeeded in a number of cases in detecting the organism in both sets of cultures, it occasionally occurred that it was found by only one of us in a special one. It is gratifying to say that the organism was readily and quickly isolated by both of us from the "Goodrich" cultures, since the contamination, which shrouded its fine characteristics in regard to gas and indol production, was a simple colon bacillus. This was, therefore, the highly motile organization first noted by me at Ocean Springs, and which could only have been that of Sanarelli, or, as was feared, one of the proteus family, and therefore the earliest Sanarelli organism isolated after that author. Early in our effort to detect this new bacillus it was ascertained that its symbiosis with any member of the colon group materially influenced its marked motility, the germ seemingly becoming inhibited by the product of the colon organism, both in growth and motility. Bearing this in mind, it became an easier matter to detect it, and it was my fortune to isolate it from the cultures on hand in 42 per cent.[1] Concomitantly with this work it was my especial effort to determine whether the bacillus icteroides was to be found in the blood or organs of bodies dead from *other* diseases than from yellow fever, and for this purpose I conducted at the city morgue, and more frequently at the morgue of the Hospital de Alphonso XIII, a number of necropsies (21), selecting bodies of patients who had suffered from malaria, with no suspicion of specific yellow fever, or from dysentery. With the exception of necropsy No. 6, none of these gave an organism approaching in characteristics that of Sanarelli, the majority of cases giving an admixture of colon and proteus in variety.

In this exception the organism isolated so fully met the demands of Sanarelli, in regard to its growth and physiologic aspects, that it was thought the bacillus had been found in this case of simple camp dysentery, until on the fifteenth day it was found to be quite slowly liquefying gelatin 20 per cent in combination with 1 per cent agar. Further plating in effort to eliminate any contamination to which such liquefaction may have been due was prevented by our departure from Havana. I could not differentiate the organism from that of Sanarelli when compared in living culture, or in stained preparation under the microscope, and it differed widely from the slowly liquefying proteus organisms. Its influence upon animal life I had no opportunity to test. During this time we had received notification of, and invitation to see, five cases of so-called yellow fever—one through the courtesy of a private practitioner and four in military hospitals. Of these I differed in the diagnoses of two, the one in private hospital and one at the military hospital in Regla, a suburb of Havana. In the three others I concurred in the diagnosis. Fresh blood from the carefully cleansed ear-tip was taken in sterile glass bulbs, from each case after the manner of Sternberg, the capillary tube being at once sealed hermetically. It is a matter of moment that in each of the cases which I had diagnosed as yellow fever the organism was found by both of us, and fortunately in the one in which I failed to detect it it was detected by Dr. Geddings, and vice versa. In the two not thus diagnosed it was detected by neither of us, although especial effort was made to do so. While I do not wish to anticipate a collaborated report of the technical work performed in the laboratory at Havana, I will briefly state that from the

[1] A smaller per cent than if fresh culture examinations had been possible.

organisms isolated by us from the home and foreign cultures we demanded an absolute compliance with all the requirements of Professor Sanarelli in the case of his bacillus icteroides, as follows: An indefinite growth upon gelatin without its liquefaction; the same in sterile milk without precipitation of its casein; the nonproduction of gas from the decomposition of sugar, glucose or lactose, in bouillon (here it will be mentioned that all sugar tests were made with bouillon from which all muscle sugar had been extracted by a growth of bacillus coli communis for a suitable time, for in this it was found that bacillus icteroides, as obtained from the Institut Pasteur, and from our cultures, did not attack either glucose or lactose when added); the nonproduction of indol in faintest trace when dissolved by added chloroform; finally, the impossibility of inducing the property of indol production in this organism by limiting its nutriment to proteids for several successive generations. Except for its marked motility it is impracticable, otherwise, to determine the organism from the various members of the colon and proteus group. For the same reason, and because of the incompleteness of most of the observations and experiments, so suddenly interrupted and *not yet* resumed, I can only allude to the validity of the claim made by Professor Sanarelli, that the bacillus icteroides is the *cause* of yellow fever. My failure to find it in control necropsies, with the uncertain exception of necropsy No. 6; the fact of its presence in 42 per cent of native cases, as evidenced by ourselves and by Professor Archinard,[1] of New Orleans, as well as that of its more constant presence in foreign cases in the *possibility* of 100 per cent, make this organism and the claim made for it a most important one, and while there may be some reasons to an admission of the claim it was the opinion of your commission, at the time of the interruption of its work, that it would require much time and labor in technical details to determine its true rôle in the pathogenesis of yellow fever.

In conclusion I must state that our service is deeply obligated to Gen. Fitzhugh Lee, our consul-general at Havana, for counsel and advice and assistance at all times during our stay in Havana, for it was mainly due to his urbanity and gratefulness to the Spanish authorities that we were the recipients of so much courtesy from Captain-General Blanco and Secretary-General Congosto. The courtesies of General Pansano and of Colonel Marino, of the medical staff, were gratefully received. I must add my intense satisfaction in and thorough appreciation of the collaboratory work performed by P. A. Surg. H. D. Geddings, whose versatility was often evinced in the conduct of the work.

EUGENE WASDIN,
Surgeon, U. S. M. H. S.

WASHINGTON, D. C., *November 1, 1898.*

SIR: In presenting this preliminary report of the part taken by me in the investigation into the causes of yellow fever I would beg leave to enter briefly into a review of the question up to the time that I entered upon the work in New Orleans, and subsequently in Havana, Cuba, by your direction.

It is perfectly natural that a disease so well marked in its gross pathology and clinical history should, from a very early time, have afforded a field for minute investigation long before the time that the discoveries of Pasteur, Koch, and others changed the "germ theory" of disease

[1] Personal statement of a large per cent.

into a system. With the advent of means of more correct investigation, with the discoveries of the specific cause of other infectious diseases as a guide, it was perfectly natural that a disease at once so peculiar and possessing so much interest for a large portion of the Western Hemisphere should form the subject of devoted study both in the United States and in Europe.

The alleged discoveries of Babes, Gibier, Domingos Friere, Carmona y Valle, and others have passed into almost oblivion, after having excited acrimonious controversy and having been proved to be largely errors of interpretation, due mainly to faulty laboratory technique. The labors of Sternberg were arduous, systematic, and had as a result the clearing away of many erroneous impressions, and of inviting and fixing attention on the stomach, liver, and upper portion of the intestinal tract as the probable field of future research. In the meantime much labor had been expended upon the study of the micro-organisms of the normal intestinal canal, with the result that the subject was found to be one of vast dimensions and involving at every turn the elucidation of new problems and bringing to light heretofore small but most important differences. As an instance of this, and as pregnant with the most important facts, has been the study of the bacillus coli communis, for a long time considered as a single, well-defined, normal, and harmless denizen of the intestinal canal of man and most of the lower animals. But the researches of Booker and others demonstrated that it would be impossible longer to consider this organism as a single species, and that it must be considered one of a great group, possessing many characteristics in common, but at the same time presenting many points of difference, and these points not altogether morphological or cultural, but chemical and consisting in many instances in their behavior to reagents and media and in the chemistry of the products of their growth. It was notably the constant occurrence of organisms of this group which has led to most of the announcements of the discovery of the specific organism of yellow fever; discoveries which, however, did not withstand the scrutiny to which such alleged discoveries must ever be subjected.

At the conclusion of Sternberg's investigations the organism which had most attracted his attention was one isolated in Havana, possessing some of the characteristics as then known and considered of the bacillus coli communis but lacking others, which was pathogenic to animals, and which he designated as "bacillus X."

In 1897 there were presented at the Institut Pasteur in Paris two claims to the discovery of the specific organism of yellow fever, one from Havelburg, of Rio de Janeiro, Brazil, the other from Professor Sanarelli, of the University of Montevideo, both trained observers and both pupils of the institute. Their claims were widely different, and it was obvious that one of the two claims must be rejected. This was not a matter of difficulty. Investigation showed that Havelburg had added one more to the number of those whose differentiation of the colon bacillus had not been sufficiently minute and painstaking. The claims of Sanarelli were more intricate. His theory of infection was new and elaborate, but unfortunately the number of cases in which his alleged bacillus icteroides was found was only slightly over 50 per cent.

Such was the status of the yellow fever question when I was ordered by you in October, 1897, to join P. A. Surg. Eugene Wasdin, United States Marine-Hospital Service, in New Orleans, and jointly with him to continue investigations into the etiology of the disease. On arriving in that city I found that Dr. Wasdin had collected material from numer-

ous necropsies and there was no lack of material. Our investigations were made in the bacteriological laboratory of the medical department of Tulane University, and we received much aid and many courtesies from Prof. P. E. Archinard, who at the time was engaged on the same subject. Very early it became evident that the published descriptions of Sanarelli as to the cultural appearances of his organism were vague, indefinite, and somewhat misleading. Always associated with the colon bacillus, it was a matter of great difficulty, often of impossibility, to differentiate it from that organism. Resembling it in many particulars, it lacked certain of its characteristics and possessed others of its own. Finally it was agreed that before an organism should be considered as that of Sanarelli it should have the following well-marked characteristics:

1. *Form.*—A small, rather fine bacillus, with rounded ends and no tendency to fusiform shape, 1 to 2 micromillimeters in length and about one-fourth as broad as long, occurring singly or in pairs, but never forming long chains from culture on solid media.

2. *Motility.*—Very actively motile, with individual organisms, making decided excursions, or translations, across the field of the microscope. (In this particular the bacillus icteroides of Sanarelli differs essentially from the colon bacillus, which, while motile, is sluggishly so, while the bacillus icteroides is fully as motile, or even more so, than the bacillus typhosus.)

3. *Gas production.*—In 2 per cent lactose bouillon, not previously treated by the colon bacillus, an amount of gas production not exceeding, as compared with the colon bacillus, the proportion of 1–4. In lactose and glucose bouillon, the muscle-sugar being destroyed prior to the addition of the lactose and glucose by the growth of colon bacillus, *there is absolutely no production of gas.* (This is in great contrast with the colon bacillus, which produces a fermentation in both lactose and glucose bouillon, with abundant evolution of gases, having nearly or quite a definite chemical composition.)

4. *Production of acid.*—In litmus peptone solution a slight production of acid. (This is in marked contrast to the colon bacillus and the bacillus "X" of Sternberg, in which the production of acid is very marked, and much in excess of that of Sanarelli.)

5. *Production of indol.*—The bacillus icteroides of Sanarelli, planted in Dunham's peptone solution, after twenty-four hours gives absolutely no production of indol upon the addition of dilute sulphuric acid and sodium nitrite. (This is in specially marked contrast to both the colon bacillus and the bacillus "X" of Sternberg, the production of indol in the latter being very abundant and well marked.)

6. The bacillus icteroides of Sanarelli does not coagulate milk. Specimens from various sources have been kept under observation in milk for as much as sixty days. (Various members of the colon group differ much in the length of time required to produce coagulation of milk. In some, the change is produced in twenty-four hours; in some which have been under observation nineteen days have elapsed before the change was complete.)

It is believed that these differences fully establish the fact that the bacillus icteroides of Sanarelli, while bearing a general resemblance to the colon group in morphology and cultural appearances, differs from it widely in the products of its growth and in its production of toxins.

As I have before said, much material was accumulated in New Orleans and much time was spent in the investigation of the organs and fluids from the bodies dead of yellow fever. Special attention was also given to the study for comparative purposes of cultures of the

bacillus icteroides of Sanarelli, the bacillus "X" of Sternberg, and the ordinary colon bacillus. It can be safely said that the bacillus icteroides was found in a larger percentage of cases than had been claimed by Sanarelli in his series (58 per cent). A culture which possessed much interest was one which Dr. Wasdin had isolated from the blood of a living yellow fever patient at the detention camp at Fontainebleau, Miss., and which we always referred to in conversation as "bacillus Goodrich." Subsequently its identity with the bacillus of Sanarelli was almost incontestably proved, so to Dr. Wasdin belongs the credit of having isolated and proved the first bacillus icteroides (Sanarelli) found in a genuine case of yellow fever on the North American continent.

About the middle of November, as the laboratory of the college would be required in a few days for the instruction of students, and a sufficient number of cultures from various sources having been collected, it was decided to suspend the investigations in New Orleans, and by order of the President, Dr. Wasdin and I proceeded to Havana, island of Cuba, there to continue our investigations into the etiology of yellow fever. The season of the year was a little unfortunate, as, owing to the almost entire cessation of immigration into the island on account of the war, there was very little fever in the city, and the strained political relations existing between the United States and Spain, it was difficult to get access to the various military hospitals of the city, in spite of official promises. The time, however, was by no means wasted. A laboratory was established in the same building with the United States consulate, and here the cultures collected in New Orleans were systematically studied, and such material as could be acquired in Havana was at the same time investigated. Unfortunately the New Orleans cultures had suffered from transportation and from delay in getting our work started, so they were comparatively few in number. The results, however, may be stated in brief as follows:

	Examined.	Positive.	Negative.
Cultures from—			
Spleen	2	2	0
Liver	8	6	2
Kidney	1	1	0
Blood	1	1	0
Havana cultures from blood	4	3	1
Total	16	13	3

NOTE.—The following is offered in explanation of the above results: There were originally 24 cultures secured in New Orleans and carried to Havana. On arrival there it was found that some had been broken in transportation; some had perished from drying, and all of these were rejected and no work was done on them in Havana. Surgeon Wasdin has also called my attention to the fact that the results in one case, Necropsy No. 18, were not quite conclusive and must, to a certain extent, be reworked.

Making a percentage of 76.93, in which the organisms described by Sanarelli was found. Of course it would have been desirable to have had a larger number of cases, especially in Havana, but I think it will be conceded that a small number of cases carefully and exhaustively studied have more scientific value than a large number more cursorily examined.

Experiments had been begun upon the toxins of the bacillus icteroides, both precipitated and liquid, when it became necessary to suspend the work in Havana and to return to the United States on the 15th of March, 1898, since which time further investigation has been

prevented by other duties in connection with epidemic work in the Southern States.

It is here necessary to say a few words in connection with the claim of identity of the bacillus icteroides of Sanarelli and the bacillus "X" of Sternberg, which has recently been made. It is inconceivable to my mind how such a claim can be sustained by anyone who has carefully studied and differentiated the two organisms. The bacillus "X" is coarser, longer, and stouter than the bacillus icteroides; originally quite motile when isolated in Havana several years ago, it is now an organism, hardly as motile as the ordinary colon bacillus; in its growth it produces fermentation in both lactose and glucose agar, with a gaseous product quite similar in composition to the products of the colon bacillus; it produces acid in its growth; its production of indol is well marked and excessive, and lastly it readily coagulates milk. Indeed it would seem that the bacillus "X" is simply and solely a well marked and accentuated colon bacillus. It is pathogenic, to be sure, for rabbits, guinea pigs, and other of the lower animals, but the time has long since passed when the colon bacillus can be considered as harmless and non-pathogenic.

It has also been objected that the bacillus icteroides too closely simulates the colon bacillus. To this it can only be said that in its characteristics of growth, as previously detailed in this article, there is a wide difference, nor is its similarity nearly so great as is that of the bacillus typhosus to the bacillus coli communis. The bacillus icteroides (Sanarelli) produces toxins, precipitable by ammonium sulphate, of well-marked intensity and potency, much more so than the toxins precipitated from bacillus "X" and ordinary colon bacillus.

The agglutination and arrest of motility experiments of Archinard and Woodson would seem to make the argument in favor of the pathogenicity of the bacillus icteroides all the stronger.

In concluding this preliminary and independent report which would indicate that the bacillus icteroides of Sanarelli is the specific agent in the causation of yellow fever, I would beg to recommend that opportunity be given for further experimentation on lower animals with its toxins, and with cultures if necessary, and that both be tested in connection with the antiamaryllic serum prepared according to the methods of Sanarelli.

Very respectfully,

H. D. GEDDINGS,
Passed Assistant Surgeon, U. S. M. H. S.

APPENDIX C.

REPORT OF TESTS MADE IN LOUISIANA IN THE USE OF PROFESSOR SANARELLI'S SERUM ANTIAMARYLIC AS A CURATIVE AGENT IN YELLOW FEVER.[1]

SIR: Under your instructions of September 26, 1898, to proceed to New Orleans, La., to endeavor to obtain cases of yellow fever in which to use the serum, I called upon the authorities of the Charity Hospital on October 1 and upon those in charge of Touro Infirmary in that city, and made known to them your earnest desire to have granted us the privilege of visiting cases of this disease in those institutions and of using the serum in those we thought suitable, this to be done only in consultation with the attending physician.

Prof. Ernest Lewis, vice-president of the board of regents of the Charity Hospital, after consultation with the superintendent of the institution, informed me that it was thought best to refuse your request on the ground that a test of the same serum, presented to the institution by Professor Sanarelli, was then in process, and that the limited number of cases in hospital scarcely furnished material for this test.

The authorities at the Touro Infirmary, especially Dr. Loeber, promised every facility for the use of the serum, manifesting a lively interest in the subject. At the same time they informed me that a definitely marked case of yellow fever was very rare in their hospital; that the prevailing type was so poorly marked, so slightly developed, that often it was found necessary to rely upon the fact of the presence of the disease in the community for a diagnosis. All such cases were treated very tentatively, scarcely any medicine being demanded; and in these it was thought there could be no definite results gained from serum exhibition, since they invariably recovered. Such were the cases then on hand. But I was promised instant notification of any admission of a serious type, and preparation was made to respond to such calls.

Consultation with Surgeon Carter, senior surgeon at New Orleans, on October 8, no suitable cases having been admitted to the Touro Infirmary, decided me to seek at Wilson, La., then generally infected, cases in which to test the prophylactic influence of the serum, and I proceeded there on that date. In this village of some 600 people I found the majority of the population sick or convalescent from a very mild type of fever, so much so that there seemed but little evidence of alarm. Through the courtesy of Acting Assistant Surgeon Bland, U. S. M. H. S., I saw a number of these cases, and he and other local physicians soon acquainted the people with the object of my visit. There was evidence of an indisposition to be "experimented" upon and many declined "the South American treatment." Of those who had been

[1] Reprint from Public Health Reports, November 25, 1898.

exposed, and who would probably develop the disease, there were 3 or 4 who expressed a disposition to submit to the prophylaxis, but they invariably asked for a guaranty, and when this could not be given they hesitated between the unknown remedy and the light type of the disease.

However, 2 cases of tolerably well-developed yellow fever were found willing to receive the serum treatment, and these are detailed below. In the second case the use of the serum was followed by such annoying urticarious erythema, especially after the second injection, that the apprehensions of the family were aroused, and the fact of the new treatment having produced symptoms not seen in any other cases, and of so serious an appearance, immediately became the subject of general comment, and I quickly found the serum relieved of all sympathy and invested with unknown possibilities for harm. I could get no consent to its prophylactic use.

At this time advice from Surgeon Carter directed me to Baton Rouge, La., where fever was then very rapidly spreading, and by invitation of Dr. C. McRea I visited that city on October 12, meeting and discussing with a large number of the physicians the probability of being able to use the serum. There was an expression of the liveliest interest in the subject and a disposition to render me aid, but a general conviction that they were scarcely warranted in advising the use of the serum in view of the very simple nature of the prevailing disease. In those cases developing more serious symptoms it was not deemed best to rely upon the serum. Hoping that cases might have occurred in the Touro Infirmary at New Orleans I returned to that city, and although there had been and were none suitable in that institution, I had the opportunity to see one at the United States Marine Hospital under the care of Dr. Faget. This is detailed below:

Case No. 1.—Robert Anderson; aged 50 years; a vigorous man, was taken sick at 10 p. m., October 8, 1898, with intense pain in back and limbs and a severe rigor, followed by high fever. When seen at 4 p. m. of the 9th, in consultation with Dr. Bland, the typical facies, pains and commencing icterus left no doubt that he had succumbed to the infection to which he had been freely exposed. Thus far only a mild mercurial had been prescribed, and with his consent the antiamarylic serum was used. At 6.15 p. m., 10 c. c. of serum were injected into the connective tissues of the loin; pulse 84, temperature 38.7°; much congestion of vessels of skin of face. Temperature to be taken every two hours as nearly as practicable.

At 9 p. m., temperature 39°, pulse 86, more marked flushing of the face and some sweating; at 11 p. m., temperature 38.8°, pulse 84, semi-delirious and perspiring very freely, urine free; at 1 a. m., October 10, temperature 38.6°, pulse 81, sleeping; at 3 a. m., temperature 38.4°, pulse 78, quiet; at 6 a. m., temperature 38.1°, pulse 76, urine free; at 8 a. m., temperature 37.8°, pulse 66, urine free; at 9 a. m., 10 c. c. of serum were injected into the connective tissue of loin; at 10 a. m., temperature 37.6°, pulse 70, congestion of face; at 3.40 p. m., temperature 38.3°, pulse 78, sweating profusely; at 5.45 p. m., temperature, 38.5°, pulse 78, urine free; at 7 p. m., temperature 38.6°, pulse 78; at 9.30 p. m., temperature 38.5°, pulse 74, restless; at 11 p. m., temperature 38.5°, pulse 72, slept one and one-half hours; at 1 a. m., October 11, temperature 38.3°, pulse 74, urine free; at 3 a. m., temperature 38.3°, pulse 73, sleeping; at 5.20 a. m., temperature 38.6°, pulse 75, feeling weak; at 7.10 a. m., temperature 37.8°, pulse 70; at 9 a. m., temperature 37.7°, pulse 68, injected serum 8 c. c.; at 7 p. m.,

temperature 38.4°, pulse 78, urine free; at 10.15 p. m., temperature 38.2°, pulse 76, refused nourishment; at 3 a. m., October 12, temperature 38.1°, pulse 76; at 6 a. m., temperature 38.1°, pulse 72, slept well; at 8 a. m., temperature 37.8°, pulse 75, urine free; at 5 p. m., temperature 38°, pulse 70; at 9 p. m., temperature 37.6°, pulse 68, bowels free; at 1 a. m., October 13, temperature 37.4°, pulse 68, urine free; at 6 a. m., temperature 37.2°, pulse 62, takes food; at 6 p. m., temperature 37.3°, pulse 62, well; at 7 a. m., October 14, temperature 37°, pulse 64; at 6 p. m., temperature 37°, pulse 68, discharged.

Case No. 2.—Fred. A., aged 19; a healthy young man, son of preceding patient, and much exposed to the infection, was taken sick at 6 p. m. of October 9, 1898, with a hard chill; pain in back and limbs, intense headache and nausea. He received a mustard bath and a mercurial with phenacetin. Temperature ranged from 38.5° to 39.5°, pulse 115.

At 9 a. m., 10th, he was given 12 c. c. of serum under the skin; temperature 39.3°, pulse 110; at 10 a. m., temperature 39.6°, pulse 110, and there was marked congestion of face, lips, ears, and body; at 12 m., temperature 39.6°, pulse 110; at 3.40 p. m., temperature 39.6°, pulse 110; at 6 p. m., temperature 39.2°, pulse 114, perspiring; at 8 p. m., temperature 39°, pulse 96; at 11 p. m., temperature 38.9°, pulse 98, slept well; at 1 a. m., October 11, temperature 38.7°, pulse 96; at 3 a. m., temperature 38.5°, pulse 88; at 5 a. m., temperature 38.9°, pulse 98.

Complains of much dizziness; at 8.30 a. m. gave another 12 c. c. of serum under the skin, temperature 39°, pulse 100; at 9.30, temperature 39.3°, pulse 100; at this hour the congestion of the face was extreme, and there was marked erythema; at 12 m., temperature 39.5°, pulse 105, perspiring and ptyalism; at 4.30 we were hastily summoned, and found the patient suffering extremely from the urticarious rash, face puffed and congested, temperature 40°, pulse 110, urine abundant, copious saliva; at 6 p. m., temperature 39.6°, pulse 110, phenacetin given; at 8 p. m., temperature 38.8°, pulse 102, nausea; at 10 p. m., temperature 39°, pulse 104, and vomiting; at 12 m., temperature 39.6°, pulse 110, rash disappearing; at 2 a. m., October 12, temperature 39.3°, pulse 105, urine scant; at 4.30 a. m., temperature 39.3°, pulse 105, slept well; at 6 a. m., temperature 39.3°, pulse 110; at 9 a. m., temperature 39.3°, pulse 97, perspiring; at 11 a. m., temperature 39.4°, pulse 110; at 1.30 p. m., temperature 38.8°, pulse 106; at 5.30 p. m., temperature 39.4°, pulse 90, urine scant; at 7 p. m., temperature 39.3°, pulse 98; at 9 p. m., temperature 39.4°, pulse 98; at 12.30 a. m., October 13, temperature 38.3°, pulse 88; at 4 a. m., temperature 38.1°, pulse 81, slept well; at 6 a. m., temperature 38.4°, pulse 86; at 8 a. m., temperature 38.5°, pulse 83; at 10 a. m., temperature 38.8°, pulse 82; at 12 m., temperature 38.8°, pulse 85; at 2.30 p. m., temperature 38.8°, pulse 82; at 6 p. m., temperature 38.7°, pulse 83; at 8 p. m., temperature 38.4°, pulse 82, nauseated; at 12 m., temperature 37.8°, pulse 78; at 4.30 a. m., October 14, temperature 37.6°, pulse 77; at 7 a. m., temperature 37.5°, pulse 76; at 9 a. m., temperature 37.5°, pulse 77; at 6 p. m., temperature 37.7°, pulse 80; at 10 p. m., October 15, temperature 37.2°, pulse 78; discharged.

Case No. 3.—A. B., seaman, about 35 years of age, was admitted to the isolation ward at the United States Marine Hospital at New Orleans October 13, 1898, and when seen in consultation with Dr. Faget was moribund from almost fatal suppression of urine. He was unconscious; there was hiccough, and jactitation, small weak pulse, devoid of tone. In the hope that the serum would produce a renewal of the suppressed renal function, he was given 20 c. c. serum under the skin, and although there was noticed a slight reaction in temperature from the subnormal,

the kidneys did not react, and he died of a uræmic seizure twelve hours after the administration.

One word as to the possibilities of this serum as outlined by Professor Sanarelli. Because of its not being an antitoxin, it does not tend to overcome the toxins of yellow fever produced in the system, and depends for its curative and prophylactic properties upon its germicidal influence. Hence it is argued by Professor Sanarelli that its use will be absolutely negative in cases in which an amount of toxin has been produced sufficient to destroy life. These cases he does not attempt to treat, passing them by as out of the pale of serum influence. To those who have witnessed the successful struggle of many apparently beyond prognostic hope there will at once arise the question of properly determining in such cases the fatal degree of intoxication which exists. Therefore Professor Sanarelli advises and insists upon the early use of the serum, and thus the destruction of the organism before it has elaborated the fatal proportion of its toxin. Such an exhibition of the serum invites the criticism that the mortality rate must be that of selected cases, and therefore of diminished statistical value; and in an epidemic of mild type, such as the recrudescence of this fall in Louisiana, it would be contradicted.

As to its influence in case No. 1, there was no doubt a very prompt reaction evidenced by the rise of temperature and pulse, and the great congestion of the face and skin of body. This was followed by a cessation of all pain in a few hours, and a fall of pulse and temperature to a point lower than before the administration. The second and third injections showed less marked reaction. The exhibition of the serum produced a free flow of nonalbuminous urine, some ptyalism, and free perspiration. I am free to confess that the man would have done equally well with any ordinary medication.

In case No. 2 I am satisfied that the boy would have done as well without any medication. His mental and physical condition were not improved by its use. The influence of the serum upon the vasoconstrictors was very pronounced, and caused mental and physical suffering. At first the urine was quite free, and always nonalbuminous, but on the second day became more scant, yet the skin acted very freely. The rash was very general on trunk and limbs. The face, eyelids, and ears were swollen, puffed, and bluish in color, with slightly raised patches which resembled urticaria, the general surface being erythematous with scattered and intensely itchy plaques. The reaction in this case was excessive, the temperature rising to 40°. The family requested us to desist from this treatment, and I did not object, since the pulse was intermittent and dicrotic. As to the doses in these cases, I have never observed the action of the serum, and therefore refrained from the exhibition of the full dose directed by Professor Sanarelli, that of 20 c. c., and feel satisfied that the latter dose would have caused much alarm, and probably harm, in the second case reported. I regret that I have not been able to accomplish your purpose to have a full test made of this serum, but I realized that it was more necessary to arrive at a just conclusion of its merits, based on correct data, than merely to use it on uncertain cases. A more thorough test will be made at the earliest opportunity.

Respectfully, yours,

EUGENE WASDIN,
Surgeon, U. S. M. H. S.

WASHINGTON, D. C., *November 10, 1898.*

APPENDIX D.

EFFORT TO TEST THE "SERUM ANTIAMARYLIC.

SPECIAL REPORT UPON THE EFFORT TO TEST IN SUITABLE CASES THE "SERUM ANTIAMARYLIC," DONATED TO THE UNITED STATES BY PROFESSOR SANARELLI, OF THE UNIVERSITY OF MONTEVIDEO, URUGUAY, WITH THE REQUEST THAT IT BE TESTED AT THE EARLIEST OPPORTUNITY IN SUITABLE CASES OF YELLOW FEVER.[1]

SIR: Pursuant to your instructions of August 20, 1898, to proceed to Santiago de Cuba for the purpose of testing the serum in suitable cases, both as a curative and as a prophylatic agent, I did so at my earliest opportunity, arriving in that city on August 25. Consultation with the military officials of our Government in command convinced me that the rumors prevalent in the United States of the malarial character of the prevailing fever in Santiago, rather than yellow fever, was correct. The officials of the city declared that there was no yellow fever in Santiago; therefore I refrained from visiting the city hospitals, because of the consumption of time and the inadvisability of a difference of opinion as to diagnosis in any possible cases seen there. However, the surgeon-in-chief of the military commandant of the province of Santiago assured me of the presence of the disease, as reported to him by his subordinate officers, and gave me carte blanche to enter the hospitals at both Siboney and on the island in the bay set apart for the purpose of a yellow fever camp. Owing to the rapidly progressing discontinuance of the camp at Siboney I took up my residence at the island hospital. A careful scrutiny of the number of convalescents there, and an inspection of their clinical histories, especially in regard to the pulse and temperature tracings, convinced me that while there was, in numerous cases, a condition resembling yellow fever, yet the pulse and thermographs did not fulfill the demands as enunciated by J. C. Faget;[2] that, while the pulse tracings were generally low, there was an absence of the typical "want of coordination" seen in an *ascending* or *stationary* fever at its acme, together with a *falling* pulse. There was much food for reflection, and I was finally convinced that in all of these cases, showing an intermitting type of fever, and most of them an enlarged spleen and tender liver, a malarial intoxication was present. Yet could the slow pulse, spongy gums, yellowish (not pronounced) skin and eyes, skin extravasations, depend upon yellow fever infection? I had reason to question the statement of "albumen in the urine" in some cases, since I was led to doubt the competency of the analyst. A continued study of these facts and of the cases arriving of so-called yellow fever led me to the conclusion that the *ensemble* of

[1] Reprint from Public Health Reports, November 18, 1898.
[2] Monographic sur le type et la specificité, de la Fièvre Jaune, 1875.

symptoms which had been so freely thus diagnosed was due to a very general condition of incipient or developing scorbutus, upon which there had become implanted a malarial toxæmia. To me this seemed to develop a condition of serious danger but little inferior to yellow fever itself. At no time did I see a case which warranted the use of the curative serum, since the enlarged spleens and other evidences of malarial infection, even if I had accepted the dictum of those in charge of the hospital, would, under Sanarelli's direction, have gone far to negative the results of its use. Under such circumstances I deemed it my duty to acquaint you with the conditions existing, especially since I had, before visiting this hospital and examining the patients, reported to you the favorable opportunity to fulfill your wishes in regard to this serum, as it was presented to me by the authorities.

In differing from the opinion of the officials of this hospital, I must express my grateful appreciation of their uniform courtesy to me at all times, and of the interest there was manifested in the work committed to my charge.

It is but right to say that every official courtesy was extended by Generals Lawton and Wood, and especially by Colonel Havard, the surgeon-in-chief, of the military forces occupying the province of Santiago de Cuba.

Respectfully, yours,

EUGENE WASDIN,
Surgeon, U. S. M. H. S.

Appendix E.

Havana, Cuba, *March 3, 1899.*

Sir: I have the honor to request that you will allow us to state that in regard to the production of gas through the fermentation of sugars in bouillon the conclusion advanced by us in our preliminary report is at fault, in that we state that the bacillus of Sanarelli does not ferment glucose with the production of gas.

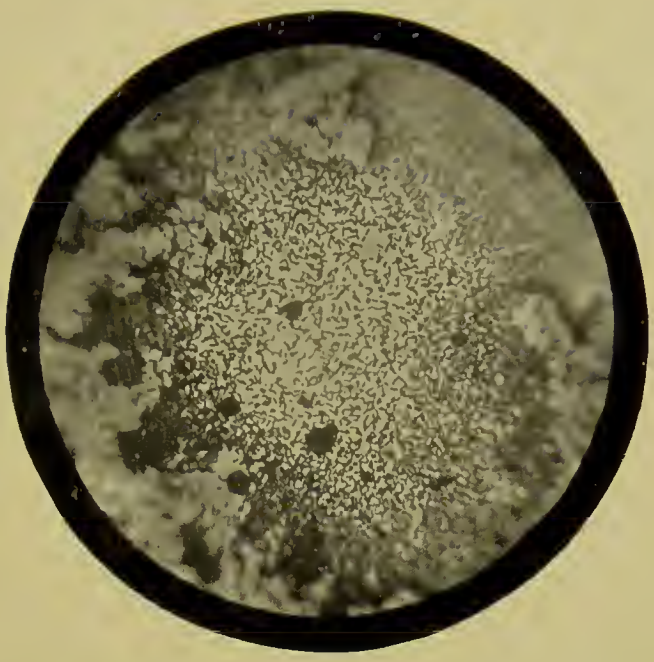
That conclusion was based upon observations actually made, the action of bacillus icteroides being noted in comparison with each organism isolated. Upon our return to this city the observation was found to have been at fault, since all examples of bacillus Sanarelli now give the production of gas with glucose. Most earnest effort was made to discover the source of this error of observation in order to correct it, and to ascertain its influence upon the other statements of our preliminary report. It suffices now to say that the fault of observation was based upon a fault of technique in the manufacture of our sugar media. This arose from the habitual use of the "Kny-Scherer" steam sterilizer, the lowest temperature in which is much too high for use in sterilizing these media. Recent experiment has demonstrated that the exposure of these sugar bouillons to the temperature of the "Arnold" sterilizer but for a short time is sufficient to cause a molecular change in the sugar, and that the usual method of sterilizing on three successive days, if done in the "Kny" cylinder, even carefully, will result in serious change in the media. Glucose under these circumstances shows less gas production, with the appropriate organism until finally this ceases. Lactose is first inverted, the organism producing now as much gas as in a glucose medium, but further heating changes it so that the production of gas ceases, although the organism, still grows freely therein. These facts depend upon the change of a part of the sugar into caramel, for in bouillon to which a saturated solution of caramel has been added in sufficient quantity, about 35 per cent, and in which Fehlings's solution shows considerable free sugar, the bacillus icteroides grows abundantly, but does not ferment the unchanged sugar. Upon the establishment of our laboratory here the lack of a good gas supply caused us to use the high-pressure sterilizer entirely, and it was more than easy to allow overheating, and since this was done with this medium in thin Smith's tubes, the change in color of the medium was not appreciated. In justice to Professor Sanarelli and other observers, and to place our report on a correct basis, we think this correction should be made at once, and not await our final report.

I have revised the work involved in the preliminary statement, and find that it is not in question otherwise.

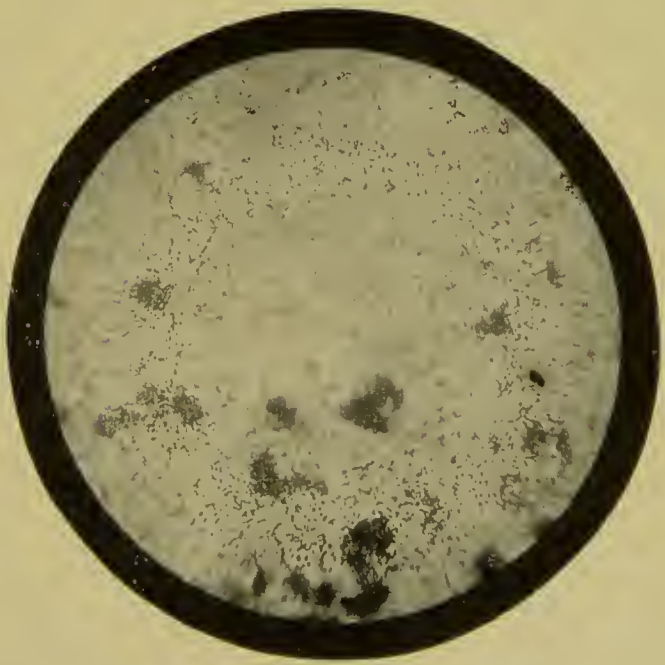
Most respectfully,

Eugene Wasdin,
Surgeon, U. S. M. H. S.

The Supervising Surgeon-General,
U. S. Marine-Hospital Service.



B. ICTEROIDES FROM AUTOPSY NO. 18, NEW ORLEANS, SHOWING AGGLUTINATION PRODUCED BY EXPOSURE OF 6 DAYS TO SERUM ANTIAMARYLL. ×500.



B. ICTEROIDES. FROM CULTURE ON AGAR-AGAR, 48 HOURS OLD. ×500.