Chapter Nine

Yellow Fever Investigations

Reports appeared in the medical literature and lay press of two phenomenal discoveries achieved independently—and nearly simultaneously—in Brazil and Mexico. In 1885, Dr. Domingos Freire, a chemist working in the medical school in Rio de Janeiro, Brazil, and Dr. Manuel Carmona y Valle in Mexico announced that they had found the agent of yellow fever and developed protective vaccines. These revelations caused a great stir among physicians in the United States, particularly along the Gulf coast. While the medical profession was divided on the veracity of these discoveries, the editor and staff of the *New Orleans Medical and Surgical Journal* lambasted Freire's abilities and experience as a pathologist and microscopist, declaring he had failed to describe or demonstrate the microbe. The following month the journal pronounced Carmona y Valle as "opposed to sound logic and accurate observation," and it also doubted his statistical acumen.[1] These discoveries were a major topic of discussion at the November American Public Health Association (APHA) meeting in Washington, DC. Dr. Joseph Holt, president of the Louisiana State Board of Health, introduced resolutions requesting that a government-sponsored investigative commission validate these new claims. Congress debated until January 1887, and then approved one government-employed physician to conduct the investigations.[2]

Although Sternberg's selection as the sole investigator was probably a foregone conclusion, Holt organized intense lobbying efforts to ensure his old friend would be named when wrangling over amendments to the bill ended. He wrote to Secretary of State Thomas F. Bayard endorsing Sternberg's candidacy in late February. In early March, Representatives Robert T. Davis (MA) and Newton C. Blanchard (LA), Senator James B. Eustis (LA), and Judge John H. Reagan (TX) presented the same endorsement directly to President Grover Cleveland, and Surgeon General John Moore added his recommendation for Sternberg by mid-April. Two weeks later, Sternberg had presidential orders in hand, his luggage packed, and a complete field outfit for bacteriological investigations prepared.[3]

Given the nature of the visit, the Sternbergs were presented at the Court of Princess Isabella, Regent of the Empire, during the absence of her father, Dom Pedro II, soon after their arrival. This point of protocol was meticulously planned, yet it appears that coordination for Sternberg's visit with Freire was somewhat faulty. Freire was in France demonstrating his inoculation technique and would not return until the first of July. The director of the medical school provided Sternberg with working space in Freire's laboratory and introduced him to two of Freire's assistants, Doctors Chapot Prevost and Joachim Caminhos. They received him warmly and provided a tour of the laboratory, but Sternberg was not impressed with the facility. Freire's microscope objectives were not state-of-the-art, and there were no culturing apparatus, dyes, solid culture media, or histological preparations. Liquid cultures were stored in three large cabinets marked "yellow fever," "cholera," and "cancer." Sternberg also learned that the yellow fever vaccine and inoculations performed in the city had divided the medical profession of Rio de Janeiro into two camps. Supporters consisted mainly of younger physicians and Freire's students, who considered the criticisms—hurled at a man they considered to be the "Pasteur of Brazil,"—to be based purely on jealousy. The opposition was composed of older physicians and leading members of the Imperial Academy of Medicine—all skeptical of their colleague's results and claims—and some had challenged his methods and use of statistics. The populace of the city was not impressed with Freire's public inoculation program either. When insufficient numbers of volunteers failed to come to his Vaccine Institute, Freire obtained government approval to vaccinate in private homes. His vaccinators invaded poor tenements by stating they were members of the board of health and claimed police authority to vaccinate by force, if necessary. The public outcry over this abuse was tremendous and supported by Freire's detractors. With this firestorm engulfing his inoculation program, it is odd that Freire chose to be absent when Sternberg arrived.[4]

Until Freire returned, Sternberg cultured the contents of flasks that supposedly contained the *Cryptococcus*, reviewed inoculation results from the preceding three years, and collected epidemiologic data from those inoculated. With the yellow fever season ending, he sought out cases and collected blood and black vomit for culture. Sternberg was given use of a culture oven in Dr. Joao Baptista de Lacerda's laboratory at the Museum of Natural History. While various bacilli grew in the Esmarch tubes he used, Sternberg could find no organism in the cultures provided or in the blood and vomit samples that fit the description of *Cryptococcus xanthogenicus*.[5]

Freire returned on July 1. When he met with Sternberg three days later, he produced a culture tube of—what he described as—pure *Cryptococcus* growing on agar-agar that he had brought back from France. Freire described in detail the growth and pathologic characteristics of the organism and how it proliferated in all organs and fluids, and he demonstrated his method of examining body fluids. To ferret out the organism in tissues, Freire pulverized the tissue in a mortar with sterile water—a process known as trituration—and then filtered the extract through a linen cloth. A drop of extract was then put on a slide with a cover slip, and observed. One can

imagine Sternberg's impressions as he watched Freire perform these primitive and obsolete laboratory techniques, but he tried to remain diplomatic. Freire was completely unfamiliar with solid media cultures and thin-section organ preparation, and when questioned about the use of aniline dyes, Freire indignantly replied he was studying the microbe in the fresh state and felt it was unnecessary to "mask them, disguise them under a costume of carnival, in order to please certain microscopists…"[6]

Sternberg determined the organism Freire presented him was *Staphylococcus aureus*—a skin contaminant—that did not fit any of the descriptions provided and produced neither spores nor pigment as Freire claimed. He concluded, "The only explanation of this wonderful versatility as to form and color…which I can conceive of is…that Dr. Freire has mistaken deformed blood corpuscles, fat globules from the liver, and the debris of tissue elements in his trituration… for micro-organisms."[7] Sternberg knew he was now on "a wild goose chase," but persevered with a thorough investigation. Blood taken from confirmed yellow fever cases in local hospitals was cultured, and tissue sections from fatal cases were examined. Of 34 inoculated culture tubes, 28 remained sterile. A variety of bacilli and micrococci grew in the remaining six tubes, a result attributed to accidental skin contamination when blood was drawn. Sternberg's tissue examinations provided an excellent example of his compulsive, exacting nature in investigational research. He noted in his report, "In all infectious diseases…due to the presence of a parasitic micro-organism in the blood, this organism may be demonstrated in properly stained thin sections of tissues. In such sections we often obtain cross-sections of small blood-vessels in which the blood corpuscles are in situ, and in which a stained micro-organism…would be very apparent."[8]

Sternberg found his Brazilian colleague's animal inoculation experiments and vaccine production to be just as imprecise and illogical as the rest of his work. Freire had inoculated monkeys, dogs, pigeons, and guinea pigs with blood from yellow fever patients all to no avail, but found injections of black vomit or cultures made from it were lethal to small rodents. It was obvious to Sternberg that the animals had died from septicemia induced by one or more of the various organisms in this material. Freire also attempted to demonstrate the lethality of the pure cultures of *C xanthogenicus* that he brought from France. First, Freire explained, the virulence of the organism, which was lost during the long voyage, had to be regenerated by injecting one gram of *Cryptococcus* bouillon culture into a pigeon. Four hours later, the regeneration was complete, the bird was killed, and one gram of cardiac blood was injected into the abdominal cavity of a guinea pig. This guinea pig survived, yet two others inoculated with unregenerated cultures died within 10 days of injection. To Freire, who concluded they died from yellow fever, this experiment vindicated his theory. Sternberg just shook his head in disbelief. What proof was there that the virulence of the cryptococcus had been reconstituted in so short a time? What proof existed that the organism injected was truly in the cardiac blood withdrawn? Cultures of this blood remained sterile. Sternberg remarked later, "Both of these guinea-pigs were supposed to have died

of yellow fever, although they had been inoculated with a culture not 'regenerated' by passing it through the blood of a pigeon, and one which he had taken with him to Paris and back. Yet he repeatedly asserts…the virulence of his microbe becomes quickly attenuated in cultures preserved for a short time…. Dr. Freire was unwilling to show me his method of inoculating man…and stated…the fact… these guinea-pigs had died was evidence…this culture—which had crossed the ocean and back—was too virulent to be used as a vaccine. Yet his experiments had been inaugurated with a view to regenerating the virulence of this same culture, upon the assumption…it was too attenuated to kill guinea-pigs."[9] To produce his vaccine, Freire injected blood from a yellow fever victim into a guinea pig or rabbit. Blood taken from this animal was injected into a second of the same species, and serial passages were repeated through six or seven animals. Blood from the last of these was cultured and serially passed at least four times in liquid culture media. The last of these cultures was used to inoculate the population of Rio de Janeiro. Again, Sternberg concluded the only reason anything grew in the culture vessels was because the original inoculum had been contaminated somewhere in the process.[10]

There was no *C xanthogenicus*. No laboratory animals had died from yellow fever and no protective vaccine had been produced. But, to be fair to Freire, Sternberg sifted through three years worth of immunization data. As he stated, "…these inoculations have been made on so large a scale, and the statistical results…appear so favorable to his method…it becomes necessary to analyze these statistics; and if… they establish the fact that the mortality from yellow fever is very much less among those who have been inoculated…than among non-inoculated persons exposed in the same way, we will be obliged to concede the value of his method, although the rationale of this protective influence may not be apparent."[11] Sternberg found Freire had little epidemiological or statistical finesse and a very poor understanding of yellow fever's natural history. Freire assumed person-to-person transmission and that the risk of disease was the same from year to year and from month to month. He failed to consider differences in exposure risk due to age, duration of residence in Rio de Janeiro, or time of year. Observation and follow-up were also inaccurate. Persons who had died from other causes or departed Rio de Janeiro before the yellow fever season were counted, and certain segments of his population were counted twice. Sternberg concluded that "…there is no satisfactory evidence that Dr. Freire's inoculations have had any prophylactic value."[12]

The Sternbergs sailed from Rio de Janeiro on August 11.[13] Mrs. Sternberg's memories of the voyage are few, other than it was dull and uncomfortable. Her husband was completely engrossed in compiling his report, and she filled in her time by making a card catalog of his notes, but the boredom was tedious. "If we were going home how happy we would both be," she said.[14] Then, although she knew he had been directed to Mexico by the president, she asked, "Why do you want to go to Mexico?"[15] Her husband looked up from his papers and gazed at her tenderly, "Because I have given so much of my time and strength to the investigation of the cause and spread of yellow fever, that I feel I have exhausted all the legitimate

experimental methods that could elucidate the subject. I hope in Mexico I can arrange to make human inoculations. In our own country this is not possible, and I now think that is the only way this problem will ever be solved."[16] Sternberg had hoped to make such experiments—transmission from person to person via blood injections—while in Brazil, but the opportunity did not present itself.

By the time the ship arrived in Barbados, quarantine authorities had been notified of smallpox epidemics in Rio de Janeiro and Pará. No one was permitted to leave the ship. After the passengers and crew were examined for evidence of smallpox and provisions were taken on, the ship continued to St. Thomas where the same rigid quarantine was in effect. Passengers could depart the ship only to go to the quarantine station where they were charged $3 per day for board and $5 per day quarantine tax that went to the station physician. Sternberg had always deplored this method of sustaining quarantine operations as nothing less than extortion. Irritated by the current quarantine system and frustrated by being trapped on a vessel moving farther away from Mexico, he could only continue with his report and hope the October 1 deadline for his investigations would be extended.[17]

Upon arrival at the quarantine station just outside of New York Harbor, he was again exasperated by what passed for disinfection. A man, who accompanied the port's deputy health officer, poured a liquid into a bucket full of some powder. He lowered it into the hold and allowed it to remain there for an hour. At the end of this time, the ship departed for the wharf. Sternberg was curious about what was in the bucket, so he asked the ship's surgeon to go with him to inspect it. They opened the hatch and hauled up the bucket. Sternberg could not detect any odor of disinfectant emanating from the hold, yet when he stuck his nose into the bucket he perceived that the reaction of liquid and powder had produced chlorine gas. He was told this small amount of material had disinfected the entire hold. Sternberg commented later, "The only object…I can conceive of depends upon the fact… there is a fee for disinfecting, which must be paid by the agents of the ship: at least I was so informed by one of the officers."[18] The Sternbergs arrived in Baltimore on September 4. He was granted an extension of 20 days for his report and departed immediately by rail for Mexico City.[19]

In Mexico City, Sternberg proceeded to the National Medical College of Mexico, where he met Carmona y Valle, director of the faculty. Sternberg was delighted with the laboratory, which contained a complete set of Robert Koch's culture apparatus, two large Zeiss microscopes with a full set of objectives, and a large English binocular microscope. It was also obvious Carmona y Valle knew how to use this equipment effectively. Carmona y Valle's yellow fever theory was based on a fungal origin. Accordingly, zoospores produced by *Peronospora lutea* damaged renal tubules, blocked urine outflow, and formed yellow spores that gave the typical skin color to disease victims. Carmona y Valle confidently demonstrated them in capillaries of liver tissue sections and provided cultures from urine of yellow fever patients. While Sternberg found Carmona y Valle's knowledge of scientific methods admirable, he also found glaring errors in its technical application. Sternberg demonstrated Carmona y Valle's spores to be masses of red blood cells

altered by preservation fluids. He determined that Carmona y Valle's cultures contained various commensal organisms from the distal urethra caused by improper collection techniques and bore no etiological relationship to yellow fever.[20]

Carmona y Valle produced his vaccine by allowing urine from yellow fever patients to evaporate in shallow plates. The residue was mixed with distilled water, and the vaccine was ready for subcutaneous injection. He inoculated himself in 1881. By November 1885, he had injected 1,358 persons in and around Mexico City. None of these people became ill with the disease, but apparently it never dawned on Carmona y Valle that his success was attributable to the fact that yellow fever did not exist in the Mexican capital, except for a few imported cases. In Vera Cruz, the story was different. In early May 1885, yellow fever struck the military garrison there, resulting in 17 cases and eight deaths. Carmona y Valle inoculated the remaining 380 soldiers and six prisoners, but of these 28 became ill and 19 died. Sternberg pointed out that these statistics, although not supportive of Carmona y Valle's method, were essentially useless. Sternberg concluded, "A simple perusal of Dr. Carmona's published work is sufficient to convince any competent bacteriologist that, owing to a defective technique and inexperience in bacteriological researches, he has fallen into serious errors of observation and of inference, and… his supposed discovery has no scientific basis."[21]

During his investigations in Vera Cruz, Sternberg met Dr. Daniel Ruiz, director of the city hospital. Ruiz vehemently denied the infectious nature of yellow fever and had no faith in Carmona y Valle's inoculations. He—like Sternberg—believed if the yellow fever agent actually resided in the blood and urine, then injecting these substances from the sick to the susceptible should produce the disease. Unfettered by moral or legal research considerations in Mexico, Ruiz had attempted such an experiment in 1885 with negative results, but gladly repeated them for Sternberg. Unfortunately, only three volunteers could be found. Two of these volunteers were injected with blood from a patient who was found at autopsy to have died of leukemia and not yellow fever. The third was injected with 50 cubic centimeters of blood from a patient with a confirmed mild case of the disease; however, as he was in the eighth day of his illness, he was beyond the period where his blood could transmit the virus, a fact unknown to Ruiz and Sternberg at the time.[22]

Sternberg returned to Baltimore in late October. At the last meeting of the APHA in Toronto, he became president of the association. His presidential address, to be given shortly in Memphis, was long, but well crafted. In this bully pulpit, he wanted to establish several objectives critical to the public health of the nation and motivate the membership to pursue them vigorously. He lamented the demise of the National Board of Health, a sound idea whose implementation had been faulty. He advocated a centrally located bureau under its own cabinet officer, directed by a commissioner with enough administrative staff to ensure efficiency and a laboratory of bacteriologists, chemists, and sanitary engineers. The commissioner would have a technical advisory board consisting of the surgeon generals of the army, navy, and marine hospital service; and presidents of state boards of health, who would have no executive power nor receive pay. Sternberg then focused

on quarantine and imported diseases. He applauded the vigilance of quarantine activities along the southern coasts, but cautioned them against complacency. No system was foolproof and, furthermore, none of the six international sanitary conferences agreed on quarantine regulations. The only way pestilential diseases could be contained within acceptable limits was through education, continual sanitary improvements, and efficient quarantine stations funded not by commerce but by the federal government and supported by the public with constant supervision by trained sanitary officers. Here is where the APHA could make an impact at the national and state levels in this area. Sternberg's vision of the APHA mission was to identify effective sanitary measures, teach community and personal hygiene, and conduct special investigations, such as the value of protective inoculations and water supply purity in various American cities and towns. Moreover, he recommended that a special fund be created by the association to encourage such investigations.[23]

Construction on the Hoagland Laboratory had begun in the summer. With Sternberg's return, plans proceeded in earnest for staffing and equipping it and developing a solid program of instruction. By mid-January 1888, however, he recognized that an assignment in New York City was unrealistic for the next two years at least. He reiterated his promise to provide not 10 but 12 weekly lectures, and on these days he also taught practical laboratory exercises for several hours. No army billets existed in New York for Sternberg, but clearly the prospect of continued government-funded yellow fever research and a functioning laboratory he could use as an operational base kept him at his Baltimore station. He was no closer to positively demonstrating the etiology of yellow fever than he had been nine years earlier. He had prepared hundreds of cultures, blood smears, and tissue sections; studied epidemiologic patterns; and modeled plausible etiologies until he was mentally exhausted. A sufficient amount of disease and cadaveric specimens—both of which were lacking in Brazil and Mexico—was required to conduct what he considered a thorough investigation, and, therefore, he would have to be in an endemic area during the epidemic season. Havana, Cuba, was the natural choice, and Sternberg began working to this end from the moment he returned from Mexico.[24]

By the time he wrote to Raymond in January, he had another strong motivation to go to Havana. Dr. Paul Gibier, a French bacteriologist, had gone to Havana in November 1887 to verify Freire's work. After demonstrating to himself that Freire was in error, he conducted his own blood studies. Finding no microorganisms in the bloodstream, Gibier then looked in the alimentary canal. The intestinal contents of many cases yielded a certain bacillus—later named *Bacillus lepina lethalis* by Sternberg—frequently enough to suggest he was on the right etiologic trail. Sternberg had closely watched Gibier's studies. He stated later, in March 1889: "The possibility that the infectious agent in yellow fever may have its habitat in the alimentary canal, occurred to me several years ago, and I determined, in advance of my visit to Havana last spring, to give special attention, to a bacteriological study of the intestinal contents."[25] In 1875, Sternberg had clearly stated his belief that the yellow fever organism—considered by him at the time to be a fungus—infected

individuals via the gastrointestinal membranes. He concluded in his *Report Upon the Prevention of Yellow Fever by Inoculation* that a thorough search of alimentary canal microorganisms was needed, but his investigations in 1879 and 1887 do not discuss it in detail, and his reports were not tremendously concerned with pathological findings in the stomach or intestines. The reason for this lies primarily in the fact that from 1879 onward he was looking for blood-borne bacteria whose major pathological impact was on the liver and kidneys. Although it is impossible to know all the things he considered in regard to his work during these extensive studies, he may have seen his primacy in the search for a yellow fever agent slipping away to a colleague who—thanks to Sternberg's previous work—knew where *not* to look for the organism. He received the desired orders to Cuba in the third week of April.[26]

Whether Gibier enthusiastically received Sternberg when he stepped off the steamer is unknown, but he gave him cultures of his newly found bacillus. By mid-May, Sternberg was convinced Gibier's organism, while lethal to laboratory animals, had nothing to do with human disease. He then scrutinized the gastrointestinal tracts of every yellow fever patient he could find, but obtaining enough autopsies was difficult. Between May 12 and June 6, he performed only 10 and had to return home because his funding ended. In his official report from 1890, he wrote, "My first five autopsies, made in 1888, gave a negative result. In case 6 [May 23], autopsy 4 hours after death, colonies of two different kinds were obtained in cultures from the blood, liver, and kidney. One of these was my bacillus a. … Again, in cases 7 and 8 the result was negative; but in case 9, in which the autopsy was made 5 hours after death, numerous colonies of bacillus a developed in my cultures from blood, liver, and kidney."[27] This version of events written more than a year later and after many experiments and much contemplation does not coincide with what Sternberg reported in his letters home. Interestingly enough, he began to see positive results in his search for a gastrointestinal agent of yellow fever almost immediately. On May 17, he wrote home in an exuberant mood: "I have some good news for you. I believe that at last I have discovered the yellow fever germ in the stomach and intestines. I have also obtained it in cultures from the kidney and urine. I will not attempt to give you particulars but there are several good reasons for believing that the bacillus which I get in my cultures is the long sought yellow fever germ…I have only had two autopsies as yet, but they were typical cases and both give me the bacillus in question…. It is not found in the blood. As I am the first one to cultivate it and to describe its characters I must be considered the real discoverer…. I am feeling very well and very cheerful at what I believe to be a successful search."[28] Four days later, he wrote home again in the same ebullient mood: "I am getting on famously and…believe I have at last discovered the yellow fever germ. I have now had three autopsies and find it in every case, not in the blood but in the stomach and intestines. It kills rabbits and guinea pigs and in a guinea pig which died on the 4th day the characteristic black liquid was in the intestines in large quantity… I am feeling very well and very happy at having accomplished that which I so long have been trying for."[29] By May 23, Sternberg

had performed six autopsies and found *Bacillus a* in all stomachs and intestines. He told his wife: "The announcement I made to you is fully confirmed and I shall publish the discovery very soon. Dr. Gibier who has been here four or five months has published the discovery of a different germ and he is wrong. I have not encountered his bacillus in any of my cases…."[30] His optimism over *Bacillus a* faded by the end of the first week in June as cultures failed to grow. In a letter home on his 50th birthday, he stated, "I can see now that I will not be able to make a definite announcement of a discovery. The best I can say is that there is some probability that my Bacillus A is the yellow fever germ. I shall have a lot of work to do again after my return home."[31] Some probability went to zero in July after he conducted an extended series of comparative experiments at Johns Hopkins. Sternberg demonstrated that *Bacillus a* was identical to the *Bacillus coli* commune of Escherich, a resident of healthy intestines worldwide and what is known as *Escherichia coli*.[32]

In his final report, one sees the steady, methodical, precise researcher. Sternberg had had time to conduct further trials, compared the results, pinpointed errors, and evaluated the whole objectively in his Baltimore laboratory. In fact, he experimented with Gibier's *Bacillus* well into December 1888. Standing in stark, uncharacteristic contrast to this is his rapid rejection of Gibier's work and the equally rapid acceptance of his own. Based on material derived from only three autopsies and the deaths of a few laboratory animals, Sternberg consigned Gibier's *Bacillus* to the trash heap of scientific history and replaced it with *Bacillus a*. Only 10 autopsies were performed during his eight weeks in Havana and, although he did not find Gibier's *Bacillus* in any of them and found *Bacillus a* in only three, this confidence in such little data defies the proper scientific conservatism that Sternberg always touted as prudent in research. Moreover, he was prepared to publish the results derived from this meager information immediately. His almost adolescent gloating over his colleague's error and his own discovery is also uncharacteristic. It may well represent—just as in his argument with Dr. Mallet in 1881—not only a self-styled priority of ownership in regard to yellow fever studies, but also a sense that, by virtue of his lengthy research on the subject, the right of discovery was reserved for George Sternberg.

In searching for the cause of yellow fever Sternberg, Gibier, Burgess, and their Cuban colleagues—Carlos Finlay, Claudio Delgado, Fernandes Malo, and others—discussed treatment modalities at length. Contemporary active therapy, including emetics, purgatives, quinine, and calomel (mercuric chloride), was considered unsatisfactory at best by physicians who saw a lot of the disease. These physicians tended more and more to advocate expectant or symptomatic treatment. Sternberg approached treatment from a more physiological perspective. His remedy consisted of 150 grains of sodium bicarbonate and 0.3 grains of bichloride of mercury mixed in a quart of ice-cold water and given in a dose of one and three-quarter ounces every hour. Sternberg described the logic of this therapy: "My principal object…was to test a decidedly alkaline treatment from the outset of the attack, with a view to relieving the gastric distress and acid vomiting which is a prominent feature of cases treated by the expectant method, and…to render the highly acid

urine neutral or slightly alkaline, in the hope that secretion would be more abundant and the tendency to suppression diminished."[33] Also, he hoped this would prevent "those structural changes which give rise to passive hemorrhage from the stomach and suppression of urine—two symptoms which present themselves in a majority of the fatal cases."[34] He stated further: "Bichloride of mercury in a comparatively small amount was added…not with the idea that it would to any extent destroy the pathogenic microorganisms in the intestine, but as an antiseptic, which might be useful in preventing fermentative changes in the stomach, which would perhaps be favored by the free administration of an alkali. The idea has also occurred to me that the specific germ may possibly find a suitable nidus in the acid secretions of the stomach, and in this case the administration of an antiseptic in combination with an alkali would be the most rational treatment. Still, I have not given much weight to this idea."[35] After Sternberg departed Havana in June, 12 cases—all confirmed as yellow fever by Dr. Burgess—were treated successfully using Sternberg's therapy at the Garcini Hospital. Eight other cases treated in the same hospital by other methods were considered controls and of these five died. Sternberg was pleased, but realized the number of cases treated was too small to substantiate the value of his method and wanted a thorough trial.[36]

While Sternberg continued to search for the etiology of yellow fever in cultures and tissue preparations during the summer in Baltimore, he also lit a fire under Joseph Raymond to find a laboratory assistant for him at the Hoagland Laboratory. Courses were to begin in October and desperation was beginning to set in. Although Sternberg told him that anyone—even a student with a short course in bacteriology in Welch's laboratory—would be acceptable, none of the candidates had satisfactory credentials. This state of affairs continued until mid-July when he found a talented prospect, George T. Kemp, Ph.D., at Johns Hopkins. Kemp had studied with H. Newell Martin and William Henry Welch, and the more Sternberg talked with him, the more enthusiastic he became that Kemp was the best candidate. In a letter to Hoagland in July, Sternberg described Kemp: "He is about 27 or 28 and has been a student in different departments of the University for about eight years. I think he is a man who would help us to build up the reputation of the Hoagland Laboratory for original scientific work, and who might take my place if in a year or two if I find it necessary to resign the honorable position to which you have appointed me…I think you will find him a very well qualified and useful man."[37] Kemp interviewed successfully with Hoagland and Raymond later in the month and was appointed as associate director of bacteriology and physiology.[38]

The summer of 1888 marked the 10th anniversary of the devastating yellow fever epidemic in the Mississippi Valley. In those 10 years, the United States had been virtually free of the scourge. A large share of the credit for this situation was given to the southern public health establishment, which had matured during the 1880s through the sound leadership of men such as Joseph Holt of Louisiana, Wirt Johnson of Mississippi, and Jerome Cochran of Alabama. By 1888, all of the southern states except Florida had state boards of health. It was, therefore,

doubly unfortunate for Florida that the next large yellow fever epidemic would originate in Jacksonville.[39]

Sternberg acted on the research opportunity presented by the Jacksonville epidemic. On September 5, he received approval from the War Department to proceed to Florida. Before he could execute these orders, he became aware—either from the newspapers or directly from Cochran—that the Jacksonville outbreak had spread to Decatur, Alabama. Sternberg stated he went to Decatur rather than Jacksonville because of the higher mortality in the former city and because he knew he would get excellent support from his old friend Cochran. He arrived in Decatur on the evening of October 3 and "found that yellow fever of a most malignant type was prevailing, and…the mortality had been very great."[40] The town would suffer 154 cases and 35 deaths, which was a mortality rate of 23 percent, by the first of November. Of the 10 physicians in Decatur, nine became ill and five died. The four remaining doctors, B. F. Cross, E. J. Conyngton, W. C. Buckley, and E. M. Littlejohn, fought the disease using Sternberg's new therapy, and Littlejohn assisted him as well in a makeshift laboratory. Sternberg performed his first autopsy later that night on a 35-year-old man who had died only an hour earlier. Although during the next month he only obtained permission for two more autopsies—none of which provided any positive information—his main purpose in Decatur was to answer a question that Gibier had raised in Havana. Assuming Gibier's *Bacillus*, *Bacillus a*, or some other bacillus caused the disease, but was found only sporadically in post-mortem tissue specimens, was it also reasonable to assume that the bacillus was present in the intestines early in the disease and then, after performing its mischief, disappeared before death? To answer this question, Sternberg collected and cultured a total of 35 fecal specimens, but found nothing.[41]

The epidemic in Florida and Alabama also provided an excellent opportunity to evaluate Sternberg's treatment on a large scale, and physicians used it in both states with excellent results. In Decatur, 64 cases were treated from the beginning of their illness with a 6 percent mortality. The control group of 90 individuals not treated by this method suffered a mortality of 34 percent. From Jacksonville, a mortality of only 4.7 percent was credited to Sternberg's therapy, and many physicians agreed that the method was remarkable in preventing urine suppression. Sternberg proudly reported these glowing statistics in the medical literature, but failed to describe his methods convincingly. It was assumed that if the patient was treated and survived, he or she did so because of the treatment. Randomized double-blind, controlled therapeutic trials were unknown at the time and, while confounding was not discussed as such, Sternberg understood the concept. He realized that race, age, and gender affected infection and recovery rates, as did severity of infection, but ignored other factors, such as nursing care, previous health of the patient, and severity of infection, which he knew were important in recovery. He had not allowed Freire or Carmona y Valle to use shoddy epidemiologic techniques, but Sternberg's desire to make an impact—if only therapeutically—on yellow fever seems to have clouded his analytical judgment. Furthermore, Sternberg apparently assumed the free flow of urine equated to successful

alkalinization with bicarbonate, but no test for urine acidity was done to verify this assumption. He had constructed a rickety therapeutic bridge part way over a wide clinical chasm and then made a leap to the other side based on faith in his statistical results. Over time the ratio of recoveries to deaths using Sternberg's therapy declined dramatically, and his treatment went away.[42]

A frost followed by a hard freeze ended the Decatur outbreak. Sternberg arrived home in early November. Two weeks later, he delivered his first lecture to 200 physicians and students gathered in the lecture hall at the Hoagland Laboratory. The lecture, which was reported in the *Brooklyn Daily Eagle*, reviewed bacteriologic science from the time Robert Koch established his research methods and techniques. Sternberg said the Germans led the way and made the most progress because of the support and encouragement provided by an enlightened government. He was saddened because the United States had contributed so little to bacteriology, but closed by stating, "Let us hope that we are entering upon a new era. Here in Brooklyn private munificence has provided the means of research which the government should have provided long ago. The fault will rest with the medical profession if active workers are not found to avail themselves of the facilities provided."[43]

December was a crowded month for Sternberg. At Johns Hopkins, experiments with cultures from Havana and Decatur continued and preparations of tissue sections were made and photographed. He refined his lectures and prepared remarks for the official dedicatory ceremonies at the Hoagland Laboratory. Opening ceremonies for the laboratory commenced at 8:00 pm on December 15 with introductory remarks by Doctors Charles H. Hall, Hoagland, Sternberg, and the Honorable Joshua Van Cott, Sr. Sternberg commented that, with such a finely appointed laboratory, he saw no reason why the Americans could not achieve the glorious deeds of the French and Germans, and received warm applause. The guest speaker for the evening, Dr. H. Newell Martin, presented a history of laboratory development from the era of the Ptolemies in Egypt to the current German models. He said that previous scientific research had been government funded and controlled. Martin proudly noted—much to Sternberg's chagrin—that American laboratories were not so encumbered and, therefore, worked not for the government, but for the good of mankind![44]

Experiments conducted through December left Sternberg with no definite yellow fever organism. He lobbied once again to go to Cuba during the entire epidemic season, and orders dated February 5, 1889 directed him to return to Havana. In the last half of February in Brooklyn, he made new photomicrographs of all the organisms he had encountered during his laborious investigations of the past two years and gathered the bacteriologic equipment necessary for four and a half months of study. Hoagland and Raymond—still intent on securing Sternberg as a full-time professor of bacteriology—unsuccessfully attempted to lure him with financial and professional inducements. Sternberg's declination has never been explained. Probably for all of his irritation with the army concerning its disregard for his routine research, Sternberg was dedicated to the Medical Department, and

furthermore, it was his link to government funding for continued yellow fever research. Hoagland offered a fine salary, shares of collegiate profits, and research facilities in Brooklyn, but he could never support yellow fever research projects on the scale to which Sternberg had become accustomed. He arrived in Havana on March 16. Through the cooperation of the local Spanish government, Sternberg was given free access to both military and civilian hospitals. The auxiliary yellow fever commission had become a permanent research organization, and it provided continuity for continued Cuban-American research efforts.[45]

Of all the men on the auxiliary commission, Carlos Finlay was the most tenacious, scientifically courageous, and prescient in yellow fever research. His yellow fever studies predated the First Havana Yellow Fever Commission, but after studying Sternberg's 1879 photomicrographs, he formulated a novel hypothesis of yellow fever transmission based on the fact that "red blood globules are discharged unbroken in the hemorrhages of yellow fever. This fact taken in connection with the circumstance that those hemorrhages are often unattended with any perceptible break in the blood vessels, while…they constitute a most essential clinical symptom of the disease, led me to infer that the principal lesion of yellow fever should be sought for in the vascular endothelium. The disease is transmissible, it attacks but once the same person, and always presents in its phenomena a regular order comparable with that observed in the eruptive fevers…yellow fever should be considered as a sort of eruptive fever in which the seat of the eruption is the vascular endothelium."[46] It occurred to Finlay that, for transmission, infectious material from within a blood vessel of a yellow fever patient had to be withdrawn and transferred into the interior of a blood vessel of a nonimmune individual. Since person-to-person transmission did not occur, this had to be accomplished by some intermediate agent capable of tapping into blood vessels silently and repeatedly, an ability "the mosquito satisfies most admirably through its bite."[47] For the next two years, Finlay studied the habits of *Culex cubensis*. He noted only the female took numerous blood meals very soon after mating or else she died, and he theorized the blood was required for the development of fertilized eggs.[48] He postulated there must be a "transportable substance, which may be an amorphous virus, a vegetable or animal germ, a bacterium, etc., but…constitutes something tangible which requires it to be conveyed from the sick to the healthy before the disease can be propagated."[49] Finlay was convinced the mosquito conveyed this substance, but attempts to prove his theory during the summer of 1881 by infecting mosquitoes and inoculating five nonimmunes failed.[50]

Retrospectively, Finlay was a visionary. However, he was not the first to indict the mosquito of complicity in disease transmission. Sir Patrick Manson had reported certain developmental stages of *Filaria bancrofti* occurred in *Culex* mosquitoes in 1878.[51] Manson believed, however, that filaria were not transmitted by the mosquito's bite, but, upon the mosquito's death, escaped into the surrounding water. Individuals were infected when they consumed this water. At the time of Finlay's presentation, direct disease transmission from vector to human was too large of a leap of faith for many of his contemporaries. The devil was in the details

of his hypothesis and experimentation. Finlay, like Sternberg, was searching for an etiologic agent that fit the bacteriologic construct of the era. He assumed the mosquito transmitted the disease agent via a contaminated proboscis, the natural hypodermic needle it uses to obtain a blood meal, found a contaminating organism, and assumed it came from the blood of yellow fever patients.

Sternberg studied cultures sent by Finlay in the winter of 1887–1888. He had seen this organism occasionally in stomach and intestinal contents of yellow fever patients and noted it was also a common skin contaminant of patients in Havana, Vera Cruz, and Rio de Janeiro. Finlay also assumed the mosquito was competent to transmit the infection immediately after charging itself with infected blood. The yellow fever virus requires a 9–12 day incubation in the mosquito host before the infection can be transmitted. Finlay applied his mosquitoes to his volunteers within two to six days after biting a yellow fever victim. He continued these inoculations over the next eight years, but obviously they could never provide sufficient statistical significance to prove his theory. He continuously, but unsuccessfully, tried to enlist Sternberg's support for the idea of mosquito transmission. Sternberg did not consider "the subject as demanding serious attention for the reason that the mosquito does not inject the blood drawn from a yellow fever patient into the inoculated individual, but it enters the insect's stomach, and whatever remains after its meal has been digested is passed per anum. When the mosquito introduces its proboscis into the individual who is to be inoculated it is for the purpose of withdrawing blood, and it is difficult to see how any inoculation can occur, unless some virus has adhered to the exterior of the delicate instrument during the considerable interval which elapses after one full meal before the insect can be induced to fill itself again."[52] He found this possibility highly improbable. Furthermore, Ruiz' attempts in Vera Cruz to transmit yellow fever by blood injections had been negative. Although Sternberg did not consider these experiments conclusive, neither did he have any experimental evidence to show the disease agent was truly in the blood of yellow fever victims. Although Finlay and Sternberg appear to have maintained an amiable personal and professional relationship, Sternberg's a priori rejection of mosquito transmission, which meant essentially the subject was ignored in American medical circles, rankled Finlay.[53]

Upon Sternberg's arrival, the yellow fever season was just beginning, and initially cases were few and sporadic. Therefore, he studied the bacterial flora of Havana's sewers, not because he expected to find anything definitive, but because, as he said, "it [was] good preliminary work."[54] He corresponded frequently with Franklin Mall, who was studying anaerobic organisms in the Johns Hopkins Laboratory.[55]

In the second week of April, Sternberg read a letter by Dr. Frank Billings, director of the pathological laboratory at Nebraska State University, in the Medical Register of Philadelphia, which got his full attention. Billings had been studying pathological sections of tissues taken from six yellow fever patients that had been sent to him by Daniel Burgess in Havana; material from two cases had come from autopsies numbers 9 and 10, performed by Sternberg in 1888. The Nebraska physician claimed that he had found the organism described by Babes in 1885 "in the blood

in every section and in great numbers, every authority to the contrary" and confidently declared "against all contradiction, that in such a disease as yellow fever, where one finds one organism closely and sharply in many sections and all parts of these sections,…that that organism is the cause of the disease of which the individual died."[56] Billings had to be aware of the authority he was challenging and that authority quickly sent a letter to Mall: "Now if this is true it is a matter of great importance that I should know it," Sternberg wrote, "and if it is false the sooner I am satisfied of the fact the better for my peace of mind. You have all of my material in your hands, & Dr. Billings has given his method. Will you not take the matter up at once & give it your best attention & report to me as soon as possible. If Dr. Billings can demonstrate microorganisms by the methods he has given you ought to be able to do so by the same methods. Please show this letter to Prof Welch & say to him that I earnestly hope him to give a little time to this matter, & either to make mounts by the methods described or to examine yours & let me know his opinion. Certainly the matter is sufficiently important to claim some of his time. I want to know the truth about it as soon as possible for if you find what Billings claims to find it will have a bearing upon my further experimental work."[57] By mid-May Mall's analysis, presumably with the assistance of Welch and possibly William T. Councilman, had allayed Sternberg's apprehensions enough for him to let the issue rest for the summer. Later Sternberg demonstrated the organism Billings had identified as *Babes bacillus* was identical to his *Bacillus a* (*E coli*).[58]

The expected epidemic of yellowjack failed to materialize early. Sternberg complained to Martha that he was "not getting on at all" with his research because he had no autopsy material.[59] But he added, "it can't be long before some of the unfortunate Spanish soldiers will fall victims to yellow fever."[60] The first fatal case among the soldiery did not occur until April 23. He obtained permission for an autopsy of this patient and another five days later. From then until late August, Sternberg conducted a total of 30 autopsies and another 18 on persons dead from maladies other than yellow fever for comparison. He studied fresh and preserved specimens of kidney, liver, stomach, and intestines; prepared aerobic and anaerobic cultures of blood, urine, stomach, and intestinal fluids; identified and photographed a large number of organisms from these cultures; and injected them into laboratory animals to determine their virulence. His spirits rose and fell as his work proceeded. On May 6, he told Martha the discovery of the yellow fever organism would not be easy, but was sure that "whether I demonstrate the germ or not my work will stand as scientific work of value in this department of research."[61] A week later he lamented to her, "so far as I can see, I am no nearer a solution of the main question," and added, "I am doing my work thoroughly and, if I don't demonstrate the specific germ, it won't be for want of working faithfully by the most approved methods, and no one else is likely to make an easy discovery in the field if I have to give up in the end."[62]

About this time Sternberg found what he designated as *Bacillus X*. This organism resembled *Bacillus a* structurally, its virulence in laboratory animals far surpassed anything he expected, and it was "the most promising yet."[63] If found in a majority

of autopsies "it may turn out to be the specific microbe I have so long been in search of."[64] Experiments over the next 10 weeks continued to bolster his faith in the primacy of *Bacillus X*. "I am feeling more encouraged with reference to my Bacillus X," he wrote home at the end of July, "and I think now I will probably be able to announce it as a probable specific agent, even if I can't claim to have made a complete demonstration of it."[65] Twelve days later he determined the source of the organism's rapid lethality. "I am again quite hopeful with reference to my Bacillus X," he told his wife, "and have proved by experiment that it produces a deadly volatile ptomaine. I have collected this in distilled water from culture of Bacillus X and injected it into rabbits, which die from such injections in a few hours."[66] His optimism was understandably high, but tempered perhaps by memories of his impetuous rush to claim the prize from Gibier in 1887, for he added, "You can say to my friends who ask you that I have strong hopes…I have discovered the right germ but am not yet prepared to announce positively that this is the case."[67]

Sternberg sailed for home on August 31. For the next seven months, he pursued experiments with *Bacillus X* in the comfort of the Johns Hopkins Laboratory, had Councilman verify old and new slide preparations, and reviewed all his yellow fever research over the past three years. His *Report on the Etiology and Prevention of Yellow Fever*, submitted June 21, 1890, was a complete and all-encompassing tour de force that defined and described all that was known about the disease both from the clinical and research perspectives. Regrettably, Sternberg was unable to rule in or out *Bacillus X* as the specific etiologic agent. After years of difficult, painstaking effort, travel, and separation from Martha, he effectively concluded his work with two sentences: "The specific infectious agent of yellow fever has not been demonstrated. The most approved bacteriological methods fail to demonstrate the constant presence of any particular microorganism in the blood and tissues of yellow fever cadavers."[68] Sternberg was sorely disappointed and commented that, "No one can regret more than I do that the…etiology of yellow fever is not yet solved…but I at least have not to reproach myself with want of diligence or failure to embrace every opportunity for pursuing the research. The difficulties have proved to be much greater than I anticipated at the outset."[69]

Although Sternberg did not discover the long-sought yellow fever organism, he took some comfort in at least having been "able to exclude in a definite manner a majority of the microorganisms which I have encountered in my culture experiments, as well as those which various other investigators (Freire, Carmona, Finlay, Gibier) have supposed to be the specific cause of the disease."[70] In a world where fame is gained by making great discoveries and where there are no laurels for second place, Sternberg's yellow fever investigations, like his work with the pneumococcus, have become only a footnote in the annals of medical history. However, his summation above was accurate. Using state-of-the art methods and equipment, meticulous technique, and reasoning, he eliminated all microorganisms found by these methods as candidates for the etiology of yellow fever. It was no small feat in 1890 and virtually brought significant yellow fever research to a close until the last half of the decade.