1 Fear or Terror on Every Countenance

YELLOW FEVER

The production of yellow fever by the injection of blood-serum that had previously been through a filter capable of removing all test bacteria is, we think, a matter of extreme interest and importance.

Reed and Carroll, 1902 (1)

INTRODUCTION

In 1793, within two decades of the writing of the Constitution of the United States and the Declaration of Independence, Philadelphia experienced an outbreak of yellow fever which shredded the fabric of civil society. While the Declaration of Independence and the Constitution have stood as blueprints for the philosophical and practical bases of representative government, the understanding of yellow fever at that time was still mired in the miasma of pre-germ theory speculation.

The first case of yellow fever in the Philadelphia 1793 outbreak was recognized in August by Benjamin Rush as the "bilious remitting yellow fever" (2). As the outbreak grew, there was no consensus on its origin. Rush attributed it to "putrid coffee" which "had emitted its noxious effluvia" after being dumped on a dock. The College of Physicians was "of the opinion that this disease was imported to Philadelphia by some of the vessels which were in the port after the middle of July." The role of the mosquito as a vector for disease was not to be recognized until decades later. In the 1793 Philadelphia outbreak, "Fear or Terror was set on every

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countenance." The effect on families was devastating. In reporting the horror of the desertion of sick wives by husbands, the desertion of sick husbands by wives, and the departure of parents from sick children, Mathew Carey, another contemporary observer, noted that those actions ". . . seemed to indicate a total dissolution of the bonds of society in the nearest and dearest connexions." He commented on "the extraordinary panic and the great law of self-preservation. . ." (3). Rush reported on the exodus, "The streets and roads leading from the city were crowded with families flying in every direction for safety in the country" (2). J. H. Powell, the modern-day chronicler of the 1793 Philadelphia epidemic, noted that business languished and public administration virtually halted. With widespread sickness, over 40,000 deaths, and diminished population, the economy of the city collapsed. It was not until November 1793 that the city began to rebound, ". . .a time of recovery—of moral, psychological, intellectual reconstruction" (4).

Rush, who remained in the city, worked relentlessly, at times seeing upwards of 150 people in a day. At the end of his 1794 account of the epidemic, Rush tells of the effect on himself in a "Narrative of the state of the Author's body and mind" (2). Following the death of his sister, he wrote, ". . .my short and imperfect sleep was disturbed by distressing or frightful dreams. The scenes of these were derived altogether from sickrooms and graveyards." This courageous, indefatigable physician embodied the paradox of latter 18th-century Philadelphia, which was the site of advanced social-governmental thinking but backward in scientific-medical thinking. Beyond his medical pursuits, Rush was an advanced social thinker, a delegate to the Continental Congress, and a signer of the Declaration of Independence. He promoted improved conditions for mental patients and prisoners, promoted education, and promoted the abolition of slavery (5). Yet Rush also reflected the confusion and ignorance of infectious diseases before the advent of laboratory methods. Ascribing yellow fever to the effluvia of putrefying coffee, he treated infected individuals with powerful purging and bloodletting and considered all diseases derived from one cause, comparing the "multiplication of diseases" to polytheism (2). Unrecognized at this time was the association of microbes with infectious diseases, which would come in the next century, along with the recognition that specific insect species could be vectors for disease transmission. Elsewhere, too, outbreaks of yellow fever were seen as striking suddenly and "in an unaccountable fashion." A chronicler of epidemics of colonial America, John Duffy quoted from an outbreak in Charleston ". . . 'the Distemper raged, and the destroying Angel slaughtered so furiously with his Avenging Sword of Pestilence'. . ." (6). Thus, the metaphors of divine punishment, of an angry God, were the means of understanding the ravages of infection. The people were reduced to struggling with the effects of the epidemics: "'nothing was done but carrying medicines, digging graves, (and) carting the dead'. . ." (6).

The understanding of infectious diseases was to change dramatically in the next century, with the work of Louis Pasteur and Robert Koch establishing the germ theory. Just about a century after the 1793 yellow fever outbreak, the first understanding of viruses as filterable agents requiring living cells for propagation was established separately in the 1890s by Dmitri Ivanowski (7) and Martinus Beijerinck (8). Shortly thereafter, yellow fever was the first human virus shown to be a filterable agent (9). With the Philadelphia epidemic of 1793 as a dramatic backdrop, the details follow of how germ theory was proven and how the concepts of viral diseases, including yellow fever, were experimentally determined.

GERM THEORY

Seeing with one's own eyes is important for understanding the causation of infectious diseases. The microscopic or submicroscopic size of microbes was the root cause of centuries of misunderstanding of infectious diseases. For millennia, diseases were conceived as the work of demonic spirits, the wrath of God, or the miasmic emanations of decaying matter (10). These "invisible" microbes spawned massive epidemics and fear (Fig. 1). The reigning theoretical concept of disease causation was that of humoralism, of an imbalance of the four humors: blood, phlegm, black bile, and yellow bile. Interventions such as bleeding and purging were designed to restore the balance of the humors. The concept originated with Hippocrates and Galen and held sway for centuries (11). It did not account for microbes as the cause of infectious illness.

That is not to say that there weren't glimmers of recognition of transmissible infectious agents. Girolamo Fracastoro (Fracastorius), whose poem about the shepherd Syphilis named that disease, wrote of its contagiousness in the 16th century (12). In his 1546 work *On Contagion*, he described germs as transmitters of disease (13), according to Garrison the first scientific statement on the nature of contagion (11). However, it was with the development of the first crucial piece of laboratory equipment, the microscope (14), that the particulate microbial nature of infectious diseases was visualized. With improved magnifying lenses introduced by Antony van Leeuwenhoek and Robert Hooke in the 17th century, it was finally possible to describe the microscopic world (15). van Leeuwenhoek called bacteria "animalcules" (Fig. 2).

In the 19th century, Louis Pasteur laid to rest the magical thinking implicit in an unseen world when he disproved the theory of spontaneous generation. This advance relied on a second crucial innovation: artificial growth medium in which microbes could visibly multiply. Pasteur's swan-necked flask contained a growth-supportive fluid, which showed turbidity when exposed to the atmosphere and remained clear and uninfected when unexposed. Further, Pasteur's studies with silkworms established the crucial concept that specific pathological conditions were associated with

FIGURE 1 *Specter of death waiting over Panama (U. J. Keppler, 1904). Yellow fever, which had been termed "the American Plague," struck Philadelphia in 1793. It later threatened the construction of the Panama Canal, as shown in this cover illustration for* Puck, *a political satire and humor magazine. (Courtesy of Beinecke Rare Book and Manuscript Library, Yale University.)*

specific causes—a concept we now take for granted (16). After years of experimentation with the silkworm diseases pébrine and flacherie, Pasteur demonstrated their causation and means of prevention by eliminating the offending microbes.

Robert Koch, the genius who laid bare the specific causes of infectious diseases, refined the tools for laboratory diagnosis of infection (Fig. 3). He markedly facilitated the viewing of microbes through a microscope with the development of a substage condenser, a lens that concentrates light from the source through the object studied. The visualization of microbes was further enhanced through the application of histological stains to differentiate the organelles from other structures in

FIGURE 2 *van Leeuwenhoek exhibiting his microscopes for Catherine of England (painting by Pierre Brissaud). Leeuwenhoek first described bacteria viewed through his early microscopes as "animalcules." (Courtesy of the Abbott Historical Archives, Leeuenhoek exhibiting his microscopes for Catherine of England, 1939.)*

specimens (17). With his development of photomicroscopic methods, Koch was able to share his observations, and it was a revelation when his first figures were published.

Koch worked to improve another crucial laboratory tool, solid culture medium. Following the observation of the growth of bacteria on sliced potatoes and with the advice of Fannie Hesse, the wife of a physician working in his lab, Koch incorporated agar into nutrient broth and so created solid medium as a means of selectively growing bacteria (14). To this day, the growth and isolation of pure bacterial cultures on agar medium remain the standard of practice for microbiological research and diagnosis.

In his work on tuberculosis, Koch approached the vexing question of etiology. Koch's postulates had their precedent in the work of Jacob Henle (18). While these tenets have evolved over time, Koch's postulates are usually understood to include the following:

1. Regular isolation of an organism from the diseased organs and absence from healthy organs
2. Growth of the organism in pure culture
3. Re-creation of disease on transmission to a susceptible host
4. Reisolation of the offending organism from the experimental host

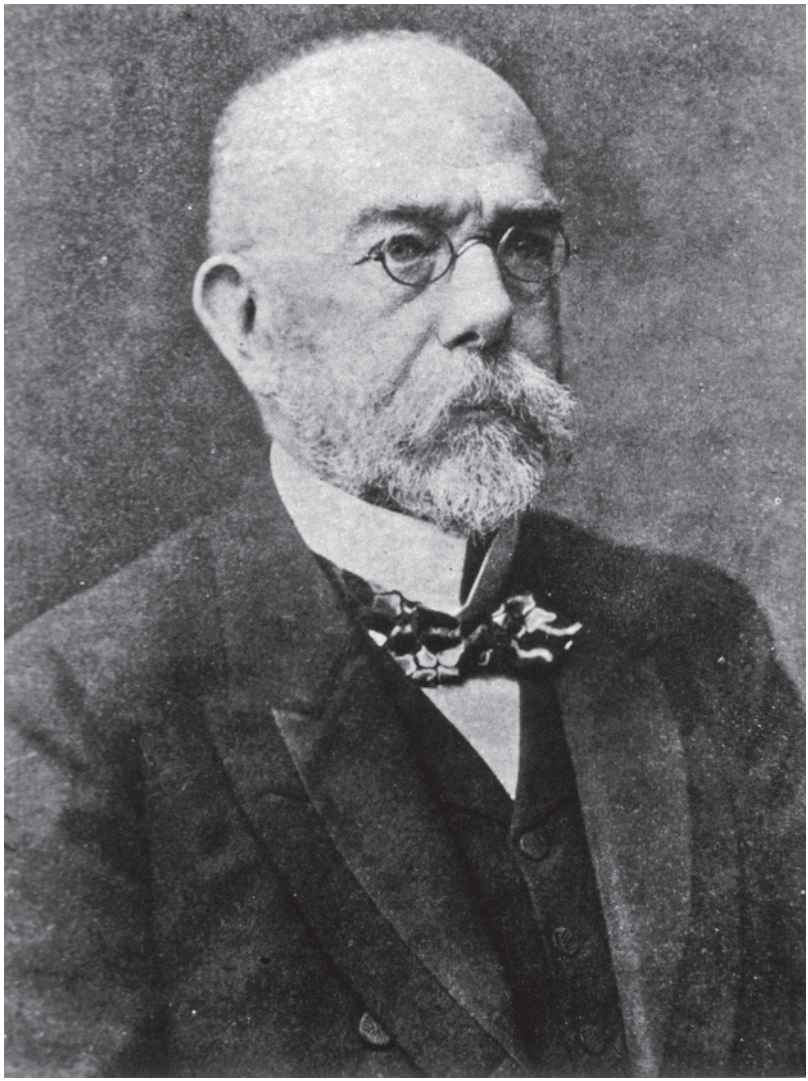

FIGURE 3 *Robert Koch, about 1908. Koch developed the methodology that allowed the emergence of bacteriology as a science. In addition, he isolated the bacteria of tuberculosis and cholera, age-old scourges of humankind. (Courtesy of the National Library of Medicine.)*

Once it was possible to demonstrate the growth of microbes on artificial media and to see these microbes microscopically, the determination of the agents of specific infectious diseases simply exploded. These developments heralded the "golden age" of microbiology between 1877 and 1906 (Table 1). Koch revealed

TABLE 1 Milestones in the golden age of bacteriology[a]

Disease	Bacterium	Year(s) isolated
Anthrax	*Bacillus anthracis*	1877
Suppuration	*Staphylococcus*, *Streptococcus*	1878, 1881
Tuberculosis	*Mycobacterium tuberculosis*	1882
Cholera	*Vibrio cholerae*	1883
Pneumonia	*Streptococcus pneumoniae*	1886
Meningitis	*Neisseria meningitidis*	1887
Gangrene	*Clostridium perfringens*	1892
Plague	*Yersinia pestis*	1904
Syphilis	*Treponema pallidum*	1902
Whooping cough	*Bordetella pertussis*	1906

[a]Modified from T. D. Brock (17), p. 290. Nomenclature retained.

the age-old scourges of civilization, "Asiatic" cholera and consumption (tuberculosis), to be associated with specific organisms. Likewise, staphylococcus and streptococcus were identified as the causes of wound suppuration, limb amputation, and frequently death during wartime and after surgical procedures. A follow-on to the recognition of lethal bacterial wound contamination was Lister's development of techniques for antiseptic surgery (19). The golden age was thus characterized by advances not only in laboratory techniques and demonstration of specific microbial causes of infections but also in the means to prevent those infections.

BIRTH OF VIROLOGY, "FILTERABLE VIRUSES"

Notably absent from the list of infectious diseases were the scourges that we now know as viral diseases. Diseases with telltale skin lesions such as disfiguring smallpox, measles, and the dramatic yellowing of malignant bilious fever or yellow fever did not succumb to isolation attempts on artificial media, nor did rage (rabies), long known for its transmission through the bite of a rabid dog. Fear of these diseases sparked scientists of the day to investigate their etiology and control. Edward Jenner's revolutionary inoculation of vesicular material from cowpox lesions blunted or prevented dreaded smallpox in recipients. Likewise, Pasteur developed attenuated rabies virus material that he used to inoculate young Joseph Meister, bitten by a vicious, rabid dog. Meister's miraculous survival unleashed a popular demand for vaccination against this frightful disease (16).

Epidemiological studies also fostered an understanding of viral disease even before viruses were actually defined and understood in the laboratory. Such was

the case when Peter Ludwig Panum followed the transmission of measles to the Faroe Islands, a group of islands in the North Atlantic Ocean. The consequences of a ship's carpenter incubating measles and delivering it to the remote Faroe Islands allowed Panum to define the transmission of this viral disease decades before the isolation and demonstration of the virus (20). In like manner, the mode of transmission of yellow fever was demonstrated before its characterization as a virus (21). That characterization depended on the defining tool of the virology laboratory; oddly enough, it was a filter customarily used to exclude bacteria. So important was that instrument that viruses were long known as "filterable viruses."

Like Pasteur's studies of silkworm diseases, the first recognition of viral diseases resulted from commercial urgency: a threat to the tobacco crop by mosaic disease. In 1885, Adolf Mayer demonstrated that infection could be transmitted by sap from diseased to healthy plants (22). In 1892, Ivanowski reported to the Academy of Science of St. Petersburg on the mosaic disease of the tobacco plant, "According to my experiments the filtered extract introduced into healthy plants produces the symptoms of the disease just as surely as does the unfiltered sap" (7). Independently, Martinus Beijerinck reported in 1898 that the tobacco disease could be transmitted through a porcelain candle filter and that tests for bacteria were negative (Fig. 4). He characterized the infection as a "*contagium vivum fluidum*," or contagious living liquid, and suggested that one form could be ". . . a contagium that exists only in living tissues . . ." (8). Hence, two laboratory requirements defined viruses: passage through a porcelain filter and the need for living cells on which to grow.

For the first demonstration of a filterable agent in mammals, again the drive was commercial. Foot-and-mouth disease impaired cattle breeding and reduced milk production, bringing severe economic hardship to Prussian agriculture (23). Friedrich Loeffler, a collaborator of Koch and the discoverer of the diphtheria bacterium and toxin, was appointed in 1897 by the Prussian Ministry of Cultural Affairs to study foot-and-mouth disease. While readily transmissible experimentally, the agent was not visible microscopically. In collaboration with Paul Frosch, Loeffler demonstrated that foot-and-mouth disease could be transmitted to cows by bacterium-free lymph which had been passed through a Berkefeld filter (24). Experiments designed to detect the presence of a toxin resulted in retention of disease-producing activity, even at extremely high dilutions. This observation raised the possibility that the effect was produced by a germ which could multiply. The hope was that a vaccine could be developed. Unfortunately, as discussed by H.-P. Schmiedebach and more recently by A. Kenubih, a vaccine with persistent immunity-inducing capacity did not result, and foot-and-mouth disease remains a scourge to this day (23, 25).

FIGURE 4 *Martinus Beijerinck in his laboratory, May 1921. Beijerinck, like Ivanowski, demonstrated that tobacco mosaic disease could be transmitted by sap which had passed through bacteriological filters. He also demonstrated the need for living cells to replicate the disease-causing factor, which he called* contagium vivum fluidum.

The work of Loeffler and Frosch stimulated work on yellow fever, a terrifying human viral disease which had been the subject of intense debate in the 19th century between the contagionists and the anticontagionists (26). Yellow fever was at once a political and commercial battle as well as a scientific matter. The politically liberal anticontagionists were against quarantines and the bureaucratic apparatus that supported quarantines. The French Academy of Medicine weighed in on the issue in 1828 against yellow fever quarantines. Thus, the matter of disease transmission had political overtones as well as commercial consequences. Walter Reed, as director of the U.S. Army Yellow Fever Commission, would definitively demonstrate the mode of yellow fever transmission. William Welch, the dean of American pathology, pointed out to Reed the possible relevance of the work of Loeffler and Frosch on foot-and-mouth disease to the etiology of yellow fever (9).

WALTER REED AND THE YELLOW FEVER COMMISSION

By 1900, several pieces of the puzzle were in place that would help to explain the spread of yellow fever. Of principal interest was the mode of transmission; the precedent had been set in 1897 with malaria when mosquitoes were identified as vectors. Walter Reed acknowledged ". . . the splendid work of Ross, Bignami, and others with regard to the propagation of malarial fever..." (9). Reed also acknowledged the work of J. C. Nott in 1848 in suggesting ". . . that the spread of yellow fever could not be assumed by the assumption of a diffusible miasm in the atmosphere but required the presence of an intermediate host. " Unfortunately, Nott, a prominent southern U.S. physician of the 19th century, also advanced racialist thinking with justification for slavery (27). The specific mosquito, then called *Stegomyia fasciata*, later called *Culex fasciatus* and finally *Aedes aegypti* (28), had been identified by Carlos Finlay in 1886. However, Finlay had failed to convince his colleagues that this mosquito was responsible for disease spread. One of the principal reasons for that failure was ignorance of an extrinsic incubation period, a time during which the virus matures in the mosquito.

Reed recognized the careful work of Henry Rose Carter (29) (Fig. 5) in two small towns in Mississippi in 1898, "'demonstrating the interval between the infecting and secondary cases of yellow fever.'" Reed was gracious in declaring, "To Dr. Carlos J. Finlay, of Havana, must be given, however, full credit for the theory of the propagation of yellow fever by means of the mosquito" (9).

Sill missing was a crucial piece of the puzzle: isolation of an agreed-upon etiological agent of yellow fever. Although this was a time of exciting discoveries in medical bacteriology, credit in disproving putative bacterial causes must go to George Miller Sternberg, a pioneer American bacteriologist. An author of early American textbooks of bacteriology in the 1890s, he spent most of his career in

FIGURE 5 *Henry Rose Carter, 1909. As a member of the Marine Hospital Service, he was able to deduce a delay between primary and secondary cases of yellow fever. This extrinsic incubation period implied the need for another, nonhuman, host, later shown to be the mosquito. He was assigned to the Panama Canal Zone in 1904 to work on yellow fever. (Courtesy of Historical Collections & Services, Claude Moore Health Sciences Library, University of Virginia.)*

the U.S. Army and was largely self-taught in bacteriology. In 1890, he published *Report on the Etiology and Prevention of Yellow Fever*, in which he thoroughly disposed of the several candidate bacteria as the cause of yellow fever (30). In 1897, Sternberg appointed Reed and James Carroll to investigate yet another candidate, *Bacillus icteroides* (Sanarelli) along with his own candidate, Bacillus X. Reed and Carroll, who were joined by Aristedes Agramonte in Cuba in 1898, demonstrated that *Bacillus icteroides* "bore no relation to the disease" (31).

In 1900, by-then Surgeon General Sternberg appointed Reed, Carroll, Agramonte, and Jesse W. Lazear to a board of army medical officers to investigate yellow fever in Cuba (31). The board first met 25 June 1900 (32). Astonishingly, within 4 months the board was able to report at the Annual Meeting of the American Public Health Association in October 1900 that *Culex fasciatus* served as the intermediate host for yellow fever (21). In clearing the field of bacterial contenders and in appointing the U.S. Army Yellow Fever Commission in 1900, Sternberg can be credited as the catalyst of these findings on the transmission and etiology of yellow fever (Fig. 6).

Without a bacterial agent identified in culture, the need remained to do studies in human subjects. The use of experimental animals was to come later (33–35). Finlay had already used human subjects in his earlier studies (36). Still, the investigators recognized the ethical implications of the studies in humans and offered themselves first. In James Carroll's words, "Then arose the question of the tremendous responsibility involved in the use of human beings for experimental purposes. It was concluded that the results, if positive, would be sufficient justification of the undertaking. It was suggested that we subject ourselves to the same risk, and this suggestion was accepted by Dr. Reed and Dr. Lazear" (31). Carroll became the first experimental subject, accepting the risks ahead of other volunteers.

The circumstances to further study yellow fever were propitious. Following the Spanish-American War, yellow fever appeared yet again in Cuba, placing the populace and American troops at risk. Soon after arrival in Cuba, Reed and his Commission colleague, Agramonte (Fig. 7), visited an army barracks at Pinar del Rio where an outbreak was occurring. Observations made on that visit ". . . did not tend to strengthen one's belief in the theory of the propagation of yellow fever by fomites" (9). A curious story was told of that visit. Only one of nine prisoners, well guarded in jail, had come down with yellow fever. Speculation was raised that an insect such as a mosquito had bitten the one prisoner. That speculation was buttressed by the observations of Carter of the interval between infecting and secondary cases (37, 38). It was decided to test Finlay's theory of mosquito transmission of yellow fever.

In Reed's words, ". . . the search for the specific agent of yellow fever while not abandoned, should be given secondary consideration, until we had first definitely

FIGURE 6 *George Miller Sternberg. Known as America's first bacteriologist, he produced the first textbook of bacteriology in the United States. He was Surgeon General of the Army from 1893 to 1902, during which time he appointed the Yellow Fever Commission. (Courtesy of the Historical Collections & Services, Claude Moore Health Services Library, University of Virginia.)*

learned something about the way or ways in which the disease was propagated from the sick to the well" (9). In preliminary experiments by Lazear, mosquito eggs were supplied by Finlay, and mosquitoes were raised in the laboratory, allowed to feed on yellow fever patients, and allowed to bite human subjects. First among the subjects was Carroll, who fell ill and almost perished (38). Lazear, apparently bitten by a stray mosquito in 1900, was a victim of their research efforts: he contracted yellow fever and died. The results of the experiments showed that 2 of 11

FIGURE 7 *The Yellow Fever Commission consisted of (upper left) Walter Reed, who led the Commission; (lower left) James Carroll, who performed the filtration experiment; (upper right) Aristides Agramonte; and (lower right) Jesse W. Lazear, who became infected in the course of the experiments and died. In a remarkably brief period of time at the turn of the 20th century, the Commission under Reed demonstrated that the disease was transmitted by mosquitoes and that it could be transmitted by filtered blood and thus was caused by a virus. (Courtesy of the Historical Collections & Services, Claude Moore Health Sciences Library, University of Virginia, except for the image of Walter Reed, courtesy of The National Library of Medicine.)*

experimentally infected subjects developed yellow fever. It was concluded that "*The mosquito acts as the intermediate host for the parasite of yellow fever, and it is highly probable that the disease is only propagated through the bite of this insect*" (italics in the original) (21).

There followed the construction of two small buildings in an open field to compare the transmission of yellow fever by fomites with transmission by the bites of infected mosquitoes or inoculation of infected blood. The "Infected Mosquito Building" was well ventilated and divided into two compartments by a screen. The "Infected Clothing and Bedding Building" was purposely not well ventilated so as to retain any noxious effects of bed clothing, pajamas, and other items from previously infected cases. After some early discouraging results, John R. Kissinger, a soldier who Reed praised for having volunteered "solely in the interest of humanity and the cause of science" and who would accept no payment, came down with experimental yellow fever from the bites of infected mosquitoes (9). In these experiments, six of seven "non-immunes" bitten by infected mosquitoes in the Infected Mosquito Building became ill with yellow fever (39). None of the seven subjects in the Infected Clothing and Bedding Building exposed to fomites from cases of yellow fever became ill, nor did subjects become ill who had remained behind the screen, not bitten by mosquitoes.

The clarity of the design of comparison groups and the results were decisive: 85.71% infected by mosquitoes versus 0% by fomites. In the definitive publication in *JAMA*, "The Etiology of Yellow Fever: an Additional Note," Reed, Carroll, and Agramonte ended with several major conclusions. In addition to confirming that "*C. fasciatus* serves as the intermediate host," they determined that 12 days or more was required after contamination for the mosquito to transmit the infection. Thus, they determined experimentally what Carter had observed epidemiologically. They found that yellow fever could be transmitted by blood subcutaneously inoculated when taken from a patient on the first 2 days of the illness. They concluded that yellow fever resulting from a mosquito bite "confers immunity" against attempted reinfection with infected blood (39).

In memory of Lazear, the experimental station established by Reed, where the crucial studies were conducted demonstrating the transmission of yellow fever by mosquitoes and not by fomites, was christened Camp Lazear. Ironically, although Carroll recovered from acute yellow fever infection, he tragically died 7 years later of myocarditis attributed to that attack of yellow fever.

An important piece of the puzzle still remained to fall in place. Walter Reed and his colleagues' final conclusion of their *JAMA* report was that ". . . the specific cause of this disease remains to be discovered" (39). Having turned away from that goal in their transmission studies, Carroll returned to the project. Initially confronted

with local objections to further experimentation, Carroll resumed his studies in September 1901 in Cuba on the nature of the infecting agent (31). In the crucial experiment, six individuals were exposed to the bites of infected mosquitoes (1). Four did not develop yellow fever, but two did. Blood was taken from patients I and II for further transmission study, but due to an accident to the vacuum pump, the blood from patient I could not be used. The blood from patient II was divided into three aliquots of partially defibrinated and diluted serum. The first aliquot, a positive control, was left untreated and successfully transmitted yellow fever to patient III. The second aliquot was heated to 55°C for 10 minutes and failed to transmit disease to patients IV, V, and VI. Based on previous work on heat stability with toxins, Reed and Carroll argued against a toxin. The third aliquot was "slowly filtered through a new Berkefeld laboratory-filter" and the filtrate was inoculated into patients VII, VIII, and IX. Patients VII and VIII developed "unmistakable" attacks of yellow fever; patient IX remained well. The scientific data were presented at the annual meeting of the Society of American Bacteriologists, 31 December 1901 and 1 January 1902. Thus, clinical virology can be said to have started in the first years of the 20th century.

Presciently, they noted that the most effective means of controlling the spread of yellow fever was through destruction of mosquito breeding areas and prevention of mosquitoes biting the sick (40). This strategy was employed with extraordinary success by William Crawford Gorgas of the U.S. Army, also present in Havana at that time. A brilliant story unto himself, Gorgas cleared Havana of yellow fever. An interesting connection can be noted here between Gorgas and J. C. Nott, mentioned above, who had suggested that yellow fever transmission required an intermediate host. Nott, coincidentally, was the doctor who delivered the infant Gorgas, whose success in the story of yellow fever eradication was based on Nott's theory. A few years later, Gorgas also cleared the Canal Zone of yellow fever and malaria, allowing the successful construction of the Panama Canal (41–43). Thus, the yellow fever-mosquito story was intimately wound into America's expanding international role (44).

It was clear from the classic studies of the Yellow Fever Commission that transmission experiments had to be performed in human subjects. However, that presented significant ethical issues, not only for potentially lethal viral infections such as yellow fever but also for permanently disabling anterior poliomyelitis.

Although Carroll reported in 1904 that others had also shown that the agent of yellow fever was filterable (31), attempts to identify a bacterial cause continued (32). It was not until a successful experimental animal host, the rhesus monkey, was demonstrated in 1928 and then the successful use of intracerebral inoculation of white mice (34, 35) that large-scale studies of the yellow fever virus could be undertaken and the bacterial candidates dismissed.

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