History of the discovery of the mode of transmission of yellow fever virus

Alan N. Clements[1] and Ralph E. Harbach[2]✉

[1]Former Emeritus Professor, London School of Tropical Medicine and Hygiene, London, UK
[2]Department of Life Sciences, Natural History Museum, London, UK, R.Harbach@nhm.ac.uk

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ABSTRACT: This essay documents and examines the historical circumstances and events surrounding the discovery of the mode of transmission of yellow fever virus in Cuba. Close scrutiny of the articles published by Walter Reed and his colleagues in 1900, 1901 and 1902 reveals their limitations as historic documents. Fortunately, other sources of information from that period survive in letters and documents written by individuals involved in the quest for the mode of transmission. Examination and comparison of those sources of information unveiled a fascinating story which reveals that misunderstandings engendered by published articles accorded merit where it was not fully due. ***Journal of Vector Ecology*** **42 (2): 208-222. 2017.**

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INTRODUCTION

Yellow fever virus, and its urban mosquito vector, both originated in Africa where a number of primate species are infected. Possibly as early as the sixteenth century, the virus and its mosquito vector were transported on ships sailing from West Africa to the West Indies. Later, due to transport by coastal shipping of both the vector and infected humans, yellow fever virus was carried to and ravaged much of the eastern regions of the Americas. Through further human associations, the urban mosquito vector of yellow fever virus became established in more eastern subtropical and tropical regions of the world, including India, Indonesia and the southern Pacific. For unknown reasons, in those regions the same mosquito species transmits dengue and chikungunya viruses to humans but not yellow fever virus (Rogers et al. 2006). The history of the circumstances and events involving the discovery of the mode of transmission of yellow fever virus in Cuba is expounded in the three sections that follow. Notes to supplementary information and unpublished letters and documents (appearing as superscript Arabic numbers in parentheses) are numbered consecutively in the text and listed at the end of Section 3.

1. Discovery of the mode of transmission of yellow fever, its remarkable history

1.1. The political and military background

Only during 1900, the last year of the nineteenth century (strictly speaking, centuries run from 01 to 100, with a new century beginning on the first day of year 01), was the mode of transmission of yellow fever established, as a result of investigations undertaken during the final decades of that century, principally on the island of Cuba. Scientific articles that reported those investigations were the source of nearly all knowledge of the subject, but almost without exception they made no mention of the political and military interactions between Spain and Cuba that brought about critical epidemiological conditions in Cuba and triggered research there. That omission was first corrected in 1989 in a work of François Delaporte: *Histoire de la fièvre jaune. Naissance de la médicine tropicale*. This was translated into English and published in 1991 as *The History of Yellow Fever: An Essay on the Birth of Tropical Medicine* (see reference for Delaporte 1989). They were followed in 2009 by Mariola Espinosa's *Epidemic Invasions*: *Yellow Fever and the Limits of Cuban Independence, 1878–1930* (Espinosa 2009), and in 2010 by S.M. Reid-Henry's *The Cuban Cure. Reason and Resistance in Global Science* (Reid-Henry 2010).

The dissatisfaction of Cubans with their harsh treatment led to the 'Ten Years War' against the Spanish from 1868 to 1878, when yellow fever reached the battle fronts during the annual summer rains. The rebellion petered out in 1878, but in 1895 a further revolt of Cuban nationalists led Spain to send 200,000 troops to Cuba, putting them at a high risk of infection.

In humans, yellow fever can vary from a mild febrile illness without jaundice to hepatorenal failure and death. In Cuba, the differences between the susceptibilities of 'native' Cubans and newly arrived Europeans to yellow fever were of great importance, the latter being much more susceptible. Finlay (1894) stated that during an epidemic in the city of Havana (La Habana to the Spanish-speaking nationals), a complete or partial acquired immunity to yellow fever was observed in all the Cuban adults and almost all the children who had been born and raised there and who had never absented themselves from that endemic focus during consecutive summers. He postulated that newborns carry an immune competence acquired *in utero* from their mothers [(1)]. Espinosa (2009) quoted a U.S. Military Hospital Service communication as reporting that during 1895 and 1896, in the port of Santiago de Cuba (Figure 1), 1,601 Spanish troops died of yellow fever but only 65 civilians died. He observed that the conventional wisdom among Cubans was that they were immune to yellow fever. Most children became infected shortly after birth, when maternal antibodies were still at a sufficient titer to protect them; later infections induced a lifelong immunity. In such situations, severe yellow fever was seen predominantly in foreigners.

During the last decades of the nineteenth century the United States had a strong interest in Cuba, principally in sugar production, amounting to $100 million annually by 1895. This involved shipping between Cuba and coastal ports of the United States. Yellow fever was endemic and epidemic in Cuba, and Havana, being a major port and a calling point on routes of coastal shipping to the United States, was a known source of yellow fever transported to the U.S. In 1878, infected passengers on steamships carrying yellow fever to New Orleans were allowed to disembark without passing through quarantine, and the yellow fever they carried was thought to have touched off the worst epidemic in U.S. history. Throughout the summer, yellow fever ravaged the population of New Orleans and spread up the Mississippi Valley as far north as St. Louis, while also moving outward along the railroad lines. Outbreaks of yellow fever occurred in more than 100 cities and towns, over 120,000 people were infected and more than 20,000 died. The economic losses across the region were estimated to amount to $100 million, possibly more (Espinosa 2009).

Experimental research into the mode of transmission of yellow fever was first undertaken in Havana by the Cuban physician Cárlos Finlay, over a period of some 25 years, which was followed by the work of a group of researchers placed in Cuba by the U.S. Army. Their experimental transmissions failed through a flaw of experimental design, which was corrected after the transfer to Cuba of an epidemiologist who had made quantitative measurements of the times of onset of primary and secondary infections of yellow fever in the southern United States (Section 1.3.2).

This essay on yellow fever covers a period that extended from 1880 in the late nineteenth century to the first decade of the twentieth century, but which in Cuba was extended by political controls. With slight modification of the accepted groupings of Cuban history as defined by Reid-Henry (2010), it falls into the following periods: (1) Spanish colonial (1492–1898), (2) United States neo-colonial (1899–1958) and (3) Socialist Cuba: (i) pre-Socialist-block collapse (1959–1991) and (ii) post-Socialist-block collapse (1992–).

For almost four centuries the Spanish had treated Cuba as a province of Spain, and when calls for independence from Spain were made in the eighteenth century they came from Cuban independence figures who "saw science as a means of achieving a free and modern Cuba" (Reid-Henry 2010). Nineteenth century examples of the promotion of science were the establishment of the *Real Academia de Ciencias Médicas, Físicas y Naturales* in Havana in 1861, after more than 35 years of negotiations with the Spanish Crown, and the endeavours of Carlós Finlay, over many years, to elucidate the mode of transmission of yellow fever (Section 1.3.1).

1.2. Active involvement of the United States

The endemicity of yellow fever in Havana and its periodic spread to the southern U.S. had long been a source of apprehension to the American people and their Government. In 1879, the U.S. sent a group of experts, the Havana Yellow Fever Commission, to Cuba to determine the sanitary conditions that allowed yellow fever to flourish in Cuban ports and to devise measures that might prevent ships bound for the U.S. from carrying the disease in any way. In 1888, George Sternberg, a U.S. Army bacteriologist who had been a member of the 1879 Commission, was sent to Cuba to investigate the biological cause of yellow fever.

In January 1898, a small riot erupted in Havana, and the battleship *USS Maine* was sent to Havana to ensure the security of American citizens and U.S. interests. On 15 February, while in Havana harbor, the *Maine* suffered a massive explosion and sank, with the loss of 266 lives. Taken to have been caused by an exploding mine, this was treated in parts of the U.S. press as due to Spanish aggression, effectively precluding a peaceful solution to the dispute, and from 21 April 1898 a state of war existed between the U.S. and Spain. The Spanish forces, worn down by years of war with Cuban insurgents and ravaged by disease, were widely overcome when faced by the fresh U.S. troops, and the fighting ceased after the city of Santiago de Cuba was taken by siege. By the end of July the war in Cuba was effectively over. The number of American soldiers killed in combat was 968, but over 5,000 died of disease, especially of yellow fever (Espinosa 2009).

On 10 December 1898, by the Treaty of Paris, Spain renounced her claims to lands discovered by Columbus, and later that month a U.S. Military Government was established in Cuba. Politically, the U.S. occupation of Cuba was above all an attempt to eliminate the source of yellow fever infections, and in the belief that yellow fever "lived in filth and in people infected with the disease" the new government put in place an extensive sanitary program. In May, 1900, with the campaign against yellow fever in Cuba failing, a Board of four medical officers (the Yellow Fever Commission) was appointed, and by direction of the Secretary of War, were to plan scientific investigations into the infectious diseases prevalent on the island (Section 2.4).

Almost a century after the loss of the *USS Maine*, examination of the structure of the wreck eliminated the possibility of any external explosion. Inspection of records of the ship's construction showed that coal bunkers surrounded the magazines and boilers. Coupled with contemporary reports of spontaneous combustion in fuel stocks in American warships that used bituminous coal, the evidence led to the conclusion that the ship could have been destroyed through a coal-bunker fire that ignited the ship's ammunition stocks (Rickover 1995, Wegner 2001, Fischer 2009).

2. Progress of studies into the mode of transmission of yellow fever, with details of the contributions of individual scientists

The quest for the mode of transmission of yellow fever occurred during the last three decades of the nineteenth century. Early reviews of progress through that time were based on original articles published by individuals engaged in that endeavor. Surprisingly, they accorded merit where it was not fully due. Those accounts were further complicated by inter-country frictions of the time. An historically based, detailed and critical study of the period and the events, entitled, as noted above, *Histoire de la fièvre jaune. Naissance de la médicine tropicale*, was written by François Delaporte and published in Paris in 1989. Only then were misunderstandings that had persisted since the early 1900s exposed. A comparable review by Espinosa, written in English, was published in 2009.

2.1. Patrick Manson (1844–1922)

The present review must necessarily start with a brief account of the work of the parasitologist Patrick Manson. He had no interest in yellow fever, but his investigations into filariasis were the first to reveal the role of mosquitoes as intermediate hosts of a

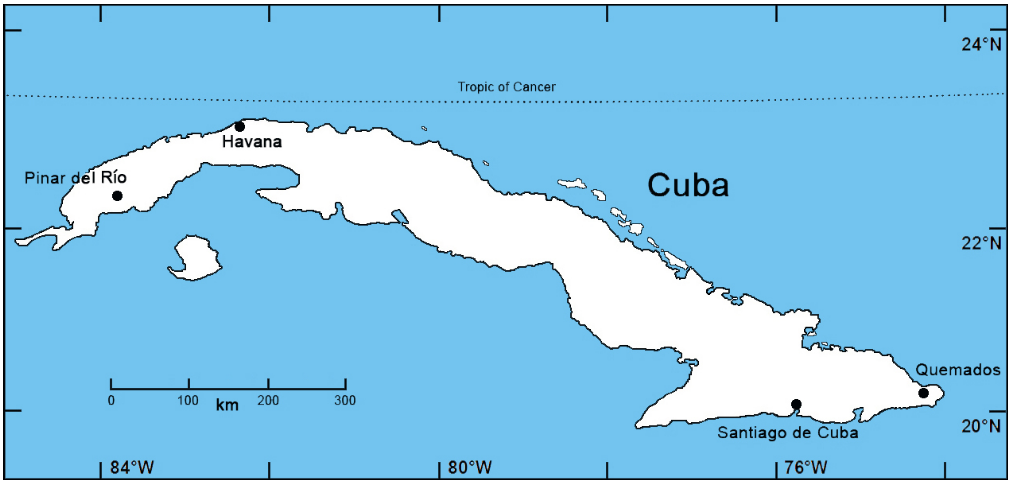

Figure 1. Outline map of the island of Cuba, showing the locations of conurbations named in the text. Havana (La Habana to the Spanish-speaking nationals), the capital city, is almost on the Tropic of Cancer (23° 26′ N), the boundary between the tropical and subtropical zones of the northern hemisphere. A major port, Havana was a calling point on routes of coastal shipping to the United States. It was the location of Military Hospital No. 1 and Las Animas Hospital. Santiago de Cuba, was the second largest harbor on the island. Pinar del Río, capital of Pinar del Río Province, was the location of Pinar del Río Barracks. Camp Columbia and Columbia Barracks Hospital were located near Quemados, then a small town. Camp Lazear was set up about one mile from the town.

pathogen, and they later influenced investigations by others into yellow fever.

From 1871 to 1883, Patrick Manson served as Medical Officer to the Chinese Imperial Maritime Customs Service, working in Amoy, a city with a Chinese population of some 200,000 on the island of Amoy off the coast of southern China. Manson undertook detailed investigations into infections with filarial nematodes, first in dogs infected with the heartworm *Filaria immitis* (now *Dirofilaria immitis*) and later in humans infected with *Filaria sanguinis hominis* (now *Wuchereria bancrofti*) (Chernin 1983). Manson reported that an average of 9% of the general population was infected, the rate increasing with age. In humans, severe cases of filariasis could cause elephantiasis, an extreme swelling of tissues of the leg or scrotum. The life cycles of these nematodes were poorly known at that time.

In the life cycle of filarial nematodes, eggs laid by adult female parasites in a mammalian host hatch within the host as largely structureless microfilariae (Manson's 'embryo filariae'). His measurements showed that in the blood of an infected dog or human there could be two million or more 'embryos' which, he realized, could not all approach the size of the mature worm without causing the death of the host. He reasoned that 'embryos' must escape from their host, possibly when taken up by a suctorial animal which fed on blood and which 'nursed' the parasites. To test his idea he procured mosquitoes that had fed on a filariasis patient and examined their abdominal contents day by day. He found filariae within blood-fed mosquitoes, some of which left the gut and developed further into first-stage stage (sausage-shaped) larvae, then into second-stage stage larvae and finally into third-stage larvae. The parasites entered mosquitoes as simple structureless animals, but left them much increased in size and with organs that were adapted for a different life style.

Manson observed that wild, blood-fed mosquitoes developed a batch of eggs within three to five days of feeding, after which they flew to bodies of water on which they laid their eggs. Most of the mosquitoes died on about the fourth or fifth day after feeding, but some survived to the sixth day. The bodies of some that died remained on the water surface, and finding that they did not contain filariae led Manson to think that any filariae formerly present had escaped into the water. Aware that cholera was contracted through drinking contaminated water, Manson had little doubt that filariae that had escaped into a water body and there came into contact with humans, either penetrated the skin or were swallowed.

These findings of Manson were submitted to *Medical Reports, China Imperial Maritime Customs* in 1877 and published in 1878 (Manson 1878), and the part of his article that was of etiological interest was published by the Linnean Society in London in 1879 (Manson 1879). Manson was the first to establish that an infectious agent present in the blood of a mammalian host could be ingested by a hematophagous arthropod, and develop and grow to a more advanced stage within the tissues of the arthropod.

2.2. Cárlos Juan Finlay (1833–1915)

Cárlos Finlay was born in Cuba of a Scottish father, a physician, and a French mother. From an early age, much of his education was in Europe and the United States. As a youngster he lived in Cuba, but from 1844–1846 and 1848–1851 he was at school in Europe, mostly in France, returning to Cuba in 1846 and 1851 for medical reasons. He received his medical training in France, Philadelphia and Havana, started to practice medicine in Matanzas, Cuba, and eventually settled in Havana, the capital city (Guiteras 1965).

2.2.1. Finlay's achievements

At 23° N, Havana is almost on the Tropic of Cancer (Figure 1). Havana was a major port, and a calling point on routes of coastal shipping which extended to the United States. Yellow fever had long been endemic on the island of Cuba, and was periodically epidemic, especially among foreigners. From his medical practice and wider responsibilities, Finlay learned much about the effects of yellow fever on the local populations, and in 1879 the Spanish government of Cuba selected him to join the newly arrived (U.S.) Havana Yellow Fever Commission, when contact with other members of the Commission broadened his knowledge of the disease.

The first methodical and scientific approach to the study of the mode of transmission of yellow fever was undertaken by Finlay after he had returned to Cuba. In a review article on yellow fever published in 1895, Finlay recalled that, in December 1880, a comparison of certain characteristics of yellow fever transmission with those needed for mosquito activity – such as the absence of both above a certain altitude and at low temperatures – led him to deduce that yellow fever could not be acquired by inhalation, ingestion or contact. He postulated that the mode of transmission might be by inoculation of disease germs by some piercing insect (algun insect puzzante) peculiar to yellow fever countries.

Finlay made extensive studies of Cuban mosquitoes, identifying possible vectors of the unknown infectious agent and examining their biology and behaviour. Two species of mosquito in Havana attracted Finlay's interest. One he identified as *Culex cubensis* Bigot, which is now recognized as *Cx. quinquefasciatus* Say. It was nocturnal, and engorged females could be found within mosquito nets, but he could never induce them to take a second blood meal. He noted that the eggs were laid in rafts (Finlay 1881a, 1886).

Finlay identified the other species as *Culex mosquito* Robineau-Desvoidy. Much later, Finlay (1902) commented that specimens he had shown to "the distinguished Cuban naturalist D. Felipe Poey" were identified as *Culex mosquito*, being similar to specimens Poey had taken to Paris in 1817 or 1820 and which Robineau-Desvoidy had later named "culex mosquito". The original description ends with the words "Indigenæ vocant *Mosquito*, sicut mihi retulit dominus Poey". From observations of the biology of mosquitoes in the region of Havana and elsewhere, he concluded that a crepuscular species such as *Culex mosquito* should be capable of transmitting the infective agent. Proof of this required only "reproduction of the disease through the mosquito", i.e. experimental transmission.

Finlay's (1886) description of *Cx. mosquito* shows it to have been remarkably similar to a mosquito already described, even to the "white lines in the figure of a two-stringed lyre" on the thorax. He added to his description: "…lately described, I am told, as '*Culex fasciatus*'". This mosquito was diurnal and crepuscular, and the females laid their eggs individually. Finlay commented that he "…was led to fix upon the Culex mosquito as the most likely … species to transmit the natural agent of yellow fever." *Culex mosquito* Robineau-Desvoidy is now commonly known as *Aedes* (*Stegomyia*) *aegypti* (Linnaeus).

Over a number of years Finlay carried out detailed investigations into the biology and behavior of *Culex mosquito*, and presented his earlier findings in an address delivered to the *Real Academia de Ciencias Médicas, Físicas y Naturales* in Havana on 14 August 1881, which was published in the *Anales* of the Society later that year (Finlay 1881a). He described the structure of the female mouthparts, giving details of the stylets, enclosed in a 'sheath' (labium), their penetration of the skin during feeding and entry into small blood vessels, the discharge of saliva and intake of blood into the stomach. Finlay accepted the view that saliva rendered the host blood more fluid, so assisting its uptake through the sucking apparatus.

Finlay also presented a hypothesis on the mode of transmission of yellow fever, a hemorrhagic infection (of intact blood cells). Noting reports that red blood cells were at times discharged intact in the hemorrhaged blood of sufferers, without apparent rupture of the blood vessels, he concluded that the vascular endothelium was the site of the principal lesion. Observing that females of *Culex mosquito* fed on humans more than once, at intervals of two or more days, he postulated that if yellow fever was contagious through the blood, a mosquito might bury its stylets in the capillaries of a yellow-fever patient at an appropriate period of the disease, contaminating its mouthparts with infective blood. Then it might later inoculate that infective blood into a person exposed to its bite.[(2)]

Finlay submitted his theory to practical tests. *Culex mosquito* females were collected in tubes while feeding on healthy individuals, and then were allowed to resume their feeding on confirmed cases of yellow fever. Two to four days later, females that had digested their blood meals were applied to the arms of healthy volunteers, whose fitness was monitored for some time afterwards. Four attempts to transmit the disease by mosquito bite were described by Finlay (1881a), and those and two further attempts were described later in greater detail (Finlay 1886). No clear cases of transmission were observed. Between June 1881 and December 1893, Finlay undertook 88 experimental transmissions, from well-marked cases of yellow fever to healthy Europeans who had spent from <1 to 7–10 years in the country. The infected individuals were bitten on the second to sixth day of the disease, and after a lapse of two or more days the mosquitoes were applied to healthy individuals. Of the 87 individuals who were observed for between 5 and 25 days after the attempted transmission, one presented a "mild albuminuric attack" and 13 "only acclimation fevers" (Finlay 1894). [Albuminuria, the presence of serum proteins in the urine, helps distinguish yellow fever from other types of viral hepatitis. Acclimation fever was fever without albuminuria.] The reasons for Finlay's failures of experimental transmission became known after findings by Henry Rose Carter (Section 2.3.2) were understood (Section 2.6.2). They had failed because the period of two or more days between infection of mosquitoes and the attempts to transmit were well short of the extrinsic incubation period.

Finlay's findings were available to contemporary readers of English after publication of his classic 1881 article (Finlay 1881a) in translation in *The New Orleans Medical and Surgical Journal* in 1882 (Finlay 1882b), and through the publication in 1886 of an updated article in *The American Journal of Medical Science* (Finlay 1886).

2.2.2. Finlay's originality questioned

Until recently it has been widely believed that Finlay was the first person to postulate the transmission of a pathogen from the blood of an infected vertebrate host to a healthy host by the feeding of a hematophagous arthropod, although by mechanical rather than biological transmission. Delaporte (1989) wrote that it is the historian's task to reveal how an individual who had advanced an important new idea formed his hypothesis, and he examined in detail the background to Finlay's (1881a) proposition that transmission of the infectious agent of yellow fever is by mosquitoes. He noted that Patrick Manson's first report on his investigations into the transmission of a filarial parasite by mosquitoes was published in China in 1878, and that a significant part of that article was republished in the *Journal of the Linnean Society* in 1879 (Manson 1878, 1879).

Could Finlay have known of Manson's findings at about that time? Delaporte (1989) thought so, identifying the source of information as articles in *The Lancet*, a medical journal published weekly in London. In an article in the 12 January 1878 issue of *The Lancet*, T. Spencer Cobbold, a helminthologist, reported that he had received from Manson both a letter, dated 27 November 1877, describing the discovery of human filariae in the stomach of mosquitoes that had fed on "hæmatozoal" patients, and a manuscript that described the developmental changes that the parasite underwent in the stomach of the mosquito (Cobbold 1878). In articles in issues of *The Lancet* dated 16 March 1878, 8 February 1879, and 15 February 1879. Sir John Fayrer reported Manson's finding of the progress of filarial larvae from the body of the mosquito into water where they were (said to be) swallowed by man (Fayrer 1979). Delaporte asserted that *The Lancet* was one of the primary sources of medical information available to Cuban physicians, and that the 12 January 1878 issue containing Cobbold's article reached Cuba in February 1878.

Delaporte pointed also to evidence of Finlay's early knowledge of Manson's findings. As early as 1881, Finlay was the principal contributor to a discussion paper on Filaria Sanguinis (Finlay 1881b). In 1882, he reported his observations of cases of filariasis in Havana during the previous January, and stated that he had confirmed Manson's 'law' of periodicity (Finlay 1882a). Despite those two examples, from textual analysis of Finlay's publications, Delaporte concluded that they were written to conceal his early knowledge of Manson's findings, and to sustain his own claim of being the first to demonstrate the mode of transmission of an infectious agent by mosquitoes, a claim long accepted in Cuba.

2.2.3. Summary

Finlay's interest in mosquitoes as possible intermediate host and vector of the infectious agent of yellow fever probably arose from knowledge of Manson's findings of mosquitoes having such a role in the transmission of filariasis. Nevertheless, he made some very important contributions to knowledge of the etiology of yellow fever. (1) He showed a local mosquito, *Culex mosquito*, to be a probable natural vector of yellow fever. (2) He published information on its structure, biology and behavior. (3) He proposed a theory of the mode of transmission of the infectious agent of yellow fever, which involved individual mosquitoes becoming infected by feeding on infected human hosts and also transmitting the infectious agent when subsequently feeding on healthy hosts. This theory was incorrect in postulating mechanical, not biological, transmission. (4) He devised an experimental protocol to test his theory of transmission which failed only because the need for a period of extrinsic incubation was not known, but which later was used successfully by others who had that additional knowledge.

2.3. Henry Rose Carter (1852–1925)

Henry Carter was born on a plantation in Virginia, and as a youth was shot in the leg when caught up in a skirmish between the Confederate Army and the "Yankees" during the Civil War. After the war he graduated as a civil engineer, but from early life he had been handicapped by poor health, and further health problems at that time led him to turn to medicine. He entered the medical school of the University of Maryland, graduating in 1879. In that year he was commissioned Assistant Surgeon in the Marine Hospital Service, and from then until 1888 he served in marine hospitals in Memphis and other southern cities that experienced epidemics of yellow fever. During that period he developed an interest in the epidemiology and control of a disease which remained with him for the rest of his life (Griffitts 1939).

2.3.1. Quarantine Officer

In 1888, Carter was assigned to serve as quarantine officer at Ship Island, Mississippi, in the Gulf of Mexico. For some ten years, at Ship Island and at subsequent quarantine postings along the U.S. Gulf Coast, he reviewed the rationale for the quarantine policies, with a view to establishing effective policies with uniform regulations.

To find the most effective and practicable method of quarantine, Carter investigated the epidemiology of yellow fever in that situation, including its 'period of incubation', i.e. the period in days between infection of a human host and appearance of the first symptoms. With no docks at the port he was obliged to sail out a mile or more to meet each incoming ship, where he would supervise and direct its disinfection. The ship was fumigated with sulphur dioxide, and the decks and other woodwork were washed with mercuric chloride. From his own observations and those of others, Carter concluded that the incubation period of yellow fever was less than six days. He introduced a regulation that the period during which all personnel on board should be detained for quarantine should start from their last possible exposure to infection, being the date of disinfection of the vessel. The length of the period of quarantine that he recommended was seven days, being the two days of the disinfection procedure plus the five days of the incubation period. From 1893 he established such a practice for the disinfection of vessels leaving Cuba and Mexico, to be undertaken either at the port of departure or *en route*, and so to shorten any period of quarantine on arrival.

These measures taken against the spread of yellow fever were not wholly successful. From 1893 to 1899 there were more than nine major epidemics of yellow fever in southern states of the United States. Carter was actively engaged in the measures to control these epidemics, and in 1898 and 1899 he was in general charge of such work by the Marine Hospital Service. Invasions of yellow fever into the U.S. declined, and eventually ended when the principal sources of urban yellow fever in the tropical and subtropical Americas had been controlled (Griffitts 1939).

2.3.2. Period of extrinsic incubation

Carter was able to carry out further studies into the epidemiology of yellow fever in the summer of 1898, during an outbreak in two isolated communities in Mississippi named Orwood and Taylor (at 34° N). By that time, he considered that "A proper inquiry is of the *space of time* which intervenes between the development of the first case which infected the environment and the development of the cases contracted from this environment."

Orwood was an agricultural community consisting almost exclusively of people living on farms which were mostly one mile or more apart. Practically all the inhabitants were susceptible to yellow fever. Taylor, then almost a hamlet, was considered less suitable, some of the houses being "close enough for aerial transmission of infection between them".

Carter observed that when a case of yellow fever occurred in an isolated farmhouse, persons who visited the house at that time did not catch the disease, but any who arrived about two weeks later could become infected. In Orwood, with the assistance of two local physicians, Carter recorded the dates of first occurrence of yellow fever infection in individuals, primary cases, and the dates of occurrence of the next infection in the same house, secondary cases. It was supposed that the secondary cases had contracted the disease from the environment, excluding fomites (objects contaminated with the infectious agent).

For a total of 12 households in Orwood and Taylor yielding data, the intervals between the observations of primary and secondary cases were mostly between two and three weeks, with one case of just 11 days and three cases of 21 or 23 days. Carter postulated that an infection of yellow fever may be conveyed directly from one sick of that disease to their environment. He considered that the period between the appearance of an infecting case and the appearance of a secondary case consists of three parts: (1) the time from development of the infecting case to the time the environment is capable of developing infection in other persons, (2) the time another person is exposed to the environment before they develop the disease, plus (3) the time from the date of exhibiting the disease to its development in the person. The sum of all three he termed the 'period of extrinsic incubation'. Reports of yellow fever transmission in ten shipping vessels, obtained from marine quarantine stations where good disinfection work was known to be done, revealed patterns of transmission similar to those occurring in houses (Carter 1900, Carter 1901a, 1901b). In a later study, he analysed many other cases of domestic transmission of yellow fever (Carter 1901c).

2.3.3. Cuba

Henry Carter's epidemiological investigations in the southern United States ended in late 1899 when he was ordered to Cuba, where yellow fever was still endemic, to serve as Chief Quarantine Officer for the Marine Hospital Service (Schultz 2009). He sailed from Port of Tampa, Florida, and arrived at Havana on about 6 March 1900.

While in Cuba, Carter discussed his findings with Cárlos Finlay, Jesse Lazear and Walter Reed, and also with Herbert Durham and Walter Myers who were visiting Havana from Liverpool, England. The information that he brought concerning the extrinsic incubation period of yellow fever, and critically of its duration, led to successful conclusion of the many years of investigation by Cárlos Finlay, and of the more recent work of other investigators, into the etiology of yellow fever (Section 2.5.1).

On 14 July 1900, Carter wrote to his wife saying "I am ordered to Washington and will I judge leave in a week or ten days … am inclined to think this ends my Cuban detail."[(3)] However, he visited Lazear in hospital in late September. A discussion with Reed might have been held in Washington and after research had started in Camp Lazear (Section 2.6.2). Carter's work took him to the Panama Canal Zone in 1904, where he served for five years. He had a highly regarded career in the U.S. Public Health Service, his final appointment being Assistant Surgeon General.

Most information about Carter's interactions with medical scientists in Cuba is recorded in digitized letters and other items of the period in the Hench-Reed Yellow Fever Collection.[(4)] Accounts of those interactions are given in Sections 2.5.1, 2.5.2, 2.6.1, and 2.6.2.

Henry Carter was an extremely modest man. His daughter, Laura Armistead Carter, described him as being very loath to talk about the influence of his work in determining the direction of the experiments of the Reed Board (the Havana Yellow Fever Commission), because he would have had to admit to determining the direction of its work. He argued that the only allowable ambition for a man was that of achievement, and that it did not matter who got the credit so long as the work was done.

2.4. The Yellow Fever Commission

As noted earlier (in Section 1.2), in May, 1900, with the campaign against yellow fever in Cuba floundering and by direction of the Secretary of War, a Board of medical officers was appointed to pursue scientific investigations into the infectious diseases prevalent on the island of Cuba. It was later named The Yellow Fever Commission.

2.4.1. Sources of information about the Commission and its activities

Formal descriptions of the experimental activities and findings of the members of the Commission were published in medical journals in 1900 and 1901. The articles, which had been written by the leader of the Commission, Major Walter Reed, listed the other members of the Commission as co-authors. Before their publication, each had been delivered orally by Reed at an American or Pan-American medical congress in Cuba or the United States. Review articles were published later by Reed (1902) and Agramonte (1915). Close scrutiny of the articles published in 1900, 1901 and 1902 reveals their limitations as historic documents (Reed et al. 1900, 1901a, 1901b). Fortunately, other sources of information from that period survive in the many letters written by those involved.[(4)] Retrieval of accurate information from this source requires that as many letters as possible relating to a particular topic are read and compared.

2.4.2. Members of the Commission, their work and findings

The members of the board were Major Walter Reed, Surgeon, in charge, and three others having the rank of Acting Assistant Surgeon – James Carroll, Aristides Agramonte and Jesse Lazear. Reed, an army surgeon, had later undertaken a pathology and bacteriology course at Johns Hopkins Hospital in Baltimore, his work there impressing Army Surgeon-General Sternberg. In 1900, Sternberg made Reed officer-in-charge of the Yellow Fever Commission.

Carroll was second in charge. An English-born Canadian, he had emigrated to America and enlisted in the U.S. Army. He attended medical lectures in different universities, earning an M.D. and assisted Major Walter Reed in the pathology laboratories of the University of Maryland. Agramonte, born in Cuba, lost his father when he was killed in battle against the Spanish. Taken to the U.S. he had completed his education there, and when appointed to the Board he was already in charge of the laboratory in Military Hospital No. 1, Havana, having a particular interest in yellow fever. Jesse W. Lazear first studied in the U.S., qualifying with an

M.D. After completing two years hospital service he spent a year in Europe, during part of which he worked at the Institut Pasteur in Paris. From 1895 he was a physician at the Johns Hopkins Hospital. There he followed Ronald Ross's accomplishments with great interest and pursued field work and experimentation on *Anopheles* mosquitoes with William S. Thayer, a fellow scientist.(5)

The first meeting of the four members of the Commission in their new capacity was on 25 June 1900 at Columbia Barracks Hospital, near Quemados (Figure 1), when they discussed their response to instructions sent in a letter dated 29 May from the Surgeon General of the Army, George M. Sternberg, to Major Reed: "You will naturally give special attention to questions relating to the etiology and prevention of yellow fever. As you are familiar with what has already been done by other bacteriologists in this field of investigation, I do not consider it necessary to give you any suggestions or detailed instructions. But it is evident that the most important question which will occupy your attention is that which relates to the etiology of this disease."(6) In 1896, an Italian bacteriologist, Giuseppe Sanarelli, claimed that he had isolated from yellow fever patients an organism which he named *Bacillus icteroides*, and currently members of the group were investigating whether *B. icteroides* (Sanarelli) was indeed the infectious agent of yellow fever.

"By tacit agreement, Major Reed was to be the director of the work, which was organized at the time as follows: Dr. Carroll, bacteriological work, Dr. Agramonte, autopsies and gross pathology, Dr. Lazear, microscopical pathology. No mosquito investigation was thought of at that time."(7) Already during May 1990, Lazear's notebook contained pathological observations on yellow fever cases, but without mention of mosquitoes.

Other individuals in the U.S. Army in Cuba provided voluntary service to Major Reed in his medical experiments. One was Private Edward Weatherwalks of the Hospital Corps, who was named with Aristides Agramonte among those awarded a Congressional Gold Medal for the Conquest of Yellow Fever.

2.5. Jesse William Lazear (1866–1900)

2.5.1. The course of his work

Lazear's earliest work for the Commission was bacteriological, but during that period three occurrences led to its change. The first of these was his contact with Henry Rose Carter, who had arrived in Cuba in early March 1900. Two accounts of their meeting differ in detail. From one it appears that Lazear met Carter on 25 June, and that on 26 June Carter sent him a printed copy of his article on his work in two Mississippi towns (Carter 1900). In a covering note he had written "I think this is about the argument I made to you yesterday and which you can, naturally, examine better when written out. As I said, to me the a-priori argument for Dr F.'s [Finlay's] theory has much in its favor and to me is more than plausible, although his observations as I read them are not convincing, scarcely corroborative" (Espinosa 2009, quoting a note sent by Carter to Lazear on 26 June 1900).

In 1924, in a discussion with two friends, details of which were recorded by Carter's daughter Laura,(8) Carter recalled his meeting with Lazear (see also Section 2.6.2). He said that he had shown Lazear an abominably printed copy of his article, and that after reading it Lazear had said "If these dates [i.e. the measured durations of the period of extrinsic incubation] are correct, it spells a living host." Carter told Lazear of his three possible explanations for the period of extrinsic incubation, which were not to be included in the published article, one being "The passage through an insect host, analogous to the passage through the tick in Texas cattle fever".

The second occurrence was the visit to Havana of two physicians from the Liverpool School of Tropical Medicine, H.E. Durham and W. Myers, when on their way to Brazil to study yellow fever. They arrived on about 17 July and left on 31 July (Espinosa 2009, quoting a note sent by Carter to Lazear on 26 June 1900). As Durham and Myers (1900) reported in the *British Medical Journal* of 8 September, they conferred with Reed, Carroll and Lazear, and also met Carter and Finlay. Their report largely concerned Carter's research (published in May 1900), which had demonstrated the interval of 14 to 21 days between the introduction of infecting cases of yellow fever to isolated properties and the onset of secondary cases, resulting from a change to the environment during that period due to an unknown infecting factor or some agent (Section 2.3.2). They commented that this, together with recent discoveries in malaria transmission, made Finlay's suggestion from 20 years earlier that the disease was spread by mosquitoes "hardly appears so fanciful" as "when the idea was first broached".

The third occurrence was an outbreak of yellow fever among soldiers at the Pinar del Río Barracks, in Pinar del Río (Figure 1), which continued despite the demonstration there that exposure to bedding and clothing of the sick was harmless. Other evidence pointed to the possibility of infection at a distance.

The Board met again on 1 August 1900, influenced, it appears, by their discussion with Durham and Myers and by the situation at Pinar del Río, and decided that the theory of mosquito transmission should be seriously considered, although discredited by the repeated failure of the transmission experiments of Finlay. In 1896, as noted above, the Italian bacteriologist Giuseppe Sanarelli claimed that he had isolated *Bacillus icteroides* from yellow fever patients. The possible identity of *B. icteroides* as the pathogenic agent had not been fully resolved and it was agreed that their work on the matter would continue. However, because Lazear had previously received training in the investigation of mosquitoes with reference to malaria and other diseases, Reed recommended that he should now investigate mosquitoes as possible vectors of yellow fever. Carroll and Agramonte would gradually learn how to handle insects while continuing with "the other work in hand".(7) A visit was soon made to Finlay. During the first week in August, Reed was recalled to Washington and could not return to Cuba until early October (Reed et al. 1900, Agramonte 1915).

2.5.2. Work with mosquitoes

Lazear entered details of his experimental work in one or more carefully written notebooks, keeping the information to himself. After Lazear's death on 25 September 1900, Walter Reed took possession of a notebook, and almost one month later, on 24 October, he addressed the Annual Meeting of the American Public Health Association, held in Indianapolis, giving a detailed account of Lazear's experiments, which could only have come from Lazear's notebook. The address was published shortly afterwards,(9) and is the source of most of the details of Lazear's investigations described here.

Lazear had conducted his experiments with mosquitoes provided by Finlay from the strain which he himself had used. Specimens that Lazear sent to the mosquito taxonomist L.O. Howard in Washington were identified as *Culex fasciatus* Fabricius. In his attempts at experimental transmission of the infectious agent of yellow fever, Lazear used Finlay's method of allowing a mosquito to feed on a yellow-fever patient, and a few days later allowing that mosquito to feed on a non-immune individual. In his early attempts to infect 11 non-immune volunteers by that method (Cases 1 to11), nine volunteers remained uninfected (Cases 1 to 9) but two (Cases 10 and 11) came down with yellow fever. Of the nine uninfected cases, seven had been bitten by mosquitoes after 2 to 8 days extrinsic incubation in the infected donor. Of the other two, one was bitten after 10 days extrinsic incubation and the other after 13 days; in both cases, the mosquitoes had fed on 'very mild' cases.

Of the two volunteers who came down with yellow fever, Case 10 was James Carroll, bitten by a mosquito which, 12 days before, had fed on a severe case of yellow fever on the second day of the disease. However, from Carroll's known movements at the hospital he might have been infected by a wild mosquito. Case 11, Private William H. Dean, had been bitten by four mosquitoes, of which two were significant: one that had fed on a fatal Case 12 days before and a second that had fed on a fatal Case 16 days before. The infection of Dean was taken to be a clear example of experimental transmission (Reed et al. 1900). The brief entries in Lazear's notebook give no indication as to whether he had been influenced by Carter's findings.

The accuracy of Agramonte's (1915) recollections of that time has been questioned.[9] Among them he recalled that he and Lazear had been so excited by that result that on about 5 September they conveyed their finding to Reed in Washington. However, at that time Lazear was secretive over his findings. On 8 September he had written to his wife saying "I rather think I am on the track of the real germ, but nothing must be said as yet, not even a hint. I have not mentioned it to a soul."[10]

Agramonte (1915) described how, on 16 August 1900, Lazear applied to himself a mosquito that ten days before had fed upon a mild case of yellow fever on the fifth day of the disease, and that he had remained in good health. He further recalled that on 13 September 1900 Lazear was working in the yellow fever ward of Las Animas Hospital, and while holding a tube containing a mosquito upon a man's abdomen a flying insect landed on his hand, and not wanting to disturb the mosquito in the tube he allowed the insect on his hand to feed. A few days later he showed the symptoms of yellow fever. During his illness, Lazear told Agramonte that his survival from the earlier mosquito attack had led him to believe that he was, to a certain extent, immune. Carter reported Carroll having had a similar conversation with Lazear at his hospital bed.[11] Lazear died on 25 September 1900.

Lazear's achievement was to have demonstrated, in a single case, transmission of the infectious agent of yellow fever from an infected to a non-immune individual through the bite of a mosquito, using Finlay's method of experimental transmission with the mosquito species *Culex fasciatus*, which Finlay had recognized as a natural vector. In Reed's published description of Lazear's work (Reed et al. 1900), Carter was credited with providing evidence for a period of extrinsic incubation, but there was no indication that Lazear had taken account of it in his final experiments.

2.5.3. The fate of Lazear's notebook

Walter Reed acquired Lazear's notebook shortly after his death, and during the following months made use of it to plan further experiments (Section 2.6.2). In a speech delivered to the American Association of Obstetricians, Gynecologists and Abdominal Surgeons (on an uncertain date), Dr. Philip Showalter Hench reported that after Reed's own death in 1902, many records and notebooks, including Lazear's notebook, disappeared from Reed's office in Washington.[12] Thereafter, the fate of the notebook remained unknown for some 30 years.

A rather cryptic note, dated 13 May 1932 and apparently written by Dr. Malloch, Librarian at the New York Academy of Medicine, recorded that in November 1931, Dr. __, an interne at the __ Hospital, had brought him Walter Reed's notebook of cases of yellow fever. It had been passed from the Medical School to the Medical Department of the Army, where he had saved it from being thrown out. The interne had asked several thousand dollars for the notebook, but towards the end of April 1932 he accepted the Library's valuation of $25.[13] When Reed's name was not found in the notebook, it was referred to a friend of his who did not see Reed's handwriting in a cursory examination of the earlier parts of the book, and concluded that this was not one of Reed's lost notebooks. The notebook remained unidentified and little or no attention was given to it.[12]

Several years later, Miss Laura Wood, who was writing a book on the life of Walter Reed, learned that there was an unidentified notebook in the library of the New York Academy of Medicine. Dr. Malloch told her that a young medical student who had been working at the Army Medical Library brought him the notebook, which he had found tossed into a trash can, and which he described as Reed's own. Through bargaining, Malloch had brought the price down to $25. Seeing the dates in the notebook, Miss Wood concluded that it might have been written by Lazear, and sent Photostat copies of four pages to Dr. Philip S. Hench[14]. A photocopy of page 40 of the notebook carried the following handwritten note in the left margin: "*This is without doubt the hand writing of my husband Dr Jesse W. Lazear. Mabel H. Lazear. Nov 30 1941*".[15]

2.5.4. The contents of Lazear's notebook

Lazear's notebook is now in the Historical Collections Library of the New York Academy of Medicine, where it is listed in the Library's Catalog as "MS. Yellow fever commission. [Case histories.] [Havana, 1900–1901], 240 p. 34.8 cm." It was examined by the first author and Philippa Colvin during 2014. It is a large book, 35.6 cm (14 in.) long and 22.4 cm (8¼ in.) wide, bound with hard covers. To proceed, it might help readers to have definitions of certain technical terms, basically the terms 'leaf' and 'page'[16] and *endpaper*,[17] here italicized. Its 120 leaves (sheets), typical of hard-back books, are sewn together along their inner sides forming a 'stack', which on either side is glued to the book's heavy jacket while standing free from the jacket's median flexible outer 'spine'. The dimensions of the stack are only marginally less than those of the book's covers. Its 240 pages are lined, in blue, and numbered sequentially at the tops of the pages. Of those pages, 104 carry handwriting and 136 are blank, some blank pages scattered among those with handwriting. In addition there are *endpapers*[17] in the notebook, not previously reported, one situated just inside the front cover and, exceptionally, two just inside the back cover. Each is a 'leaf' with front and back 'pages'. The leaves of all three *endpapers* are unlined. The front pages of the two at the back of the volume are numbered 291 and 292 and carry handwriting. This numbering is out of sequence with that of the text, which extends from page 1 to page 240.

2.5.5. The writers of Lazear's notebook

Modern studies of Lazear's notebook have been limited to the copy acquired in 1932 and kept in the Collections Library of the New York Academy of Medicine. Previous descriptions of its textual contents made no reference to experimental studies written on the two *endpages* at the back of the notebook. They are described briefly under the heading 'Block 4' below.

From study of a Photostat copy of the notebook, Hench reported that the first hundred pages were in Lazear's hand[13,19]. More precisely, it was pages 7–100. The entry on the page numbered 7 was dated 2 March 1900, and that on page 100 was dated 13 September 1900.

Previous studies of the notebook using the identities of the writers and the dates of writing revealed that it was composed sequentially of contributions by Jesse Lazear, Walter Reed and Jesse Lazear, which here are listed as Blocks 1, 2 and 3. Those identities were confirmed for this review by detailed studies of many photocopied pages from the notebook by the graphologist Carol Lowbeer. The two *endpapers* bound to the back cover and numbered 291 and 292 underwent the same scrutiny and graphological analysis and are treated here as Block 4 in Table 1.

In a letter dated 23 December 1941,[15] Hench stated that pages 7–34 in the notebook, dated 2 March to 12 May 1900, concerned 10 or 12 soldiers sick with different diseases. Among pages 37 ff., many not written on, are recorded Lazear's early observations on yellow fever cases, the first being that of King on 23 May. Hench commented that in examining mosquitoes captured in mosquito nets, "Lazear was hunting for changes in them analogous to those in malarial mosquitoes". During August there were notes on patients at Las Animas Hospital who were naturally infected with yellow fever, and also on patients who had been bitten by Lazear's mosquitoes, with their blood counts.[15] Lazear's last entry, on page 100, was dated 13 September 1900 and referred to "Guinea pig No. 1 – red, bitten that day by a mosquito".[16, 19] Shortly afterwards Lazear became fatally ill, and on 25 September he died. Pages 105–143 of the notebook, many not written on, were in the handwriting of Reed, and gave details of experiments on a soldier volunteer conducted under his instructions at Camp Lazear (Section 2.6.2). Pages 170 to 197 were again in Lazear's hand, and were his notes on 45 cases of malaria, mostly tertian, dated 18 February to 7 August 1900.[15] Scanning through Lazear's entries did not reveal any references to Carter, Durham, Myers or Reed,[18] confirming that this was a notebook for records of experimental work, not a diary.

On 28 February 1929, long after he had died, Major Walter Reed was awarded a Congressional Gold Medal for the Conquest of Yellow Fever. The same medal was awarded then to others who had worked with him, and among such names listed by Harrow (2004) were Aristedes Agramonte, a member of the Commission, and Edward Weatherwalks. Weatherwalks was in Cuba with the 7th Army occupation forces, and while serving with the Hospital Corps he volunteered to participate in Major Reed's yellow fever experiments.

2.6. Walter Reed (1851–1902)

From the early 1900s to this day, Walter Reed has commonly been credited with establishing, almost single-handedly, that the infectious agent of urban yellow fever is passed by biological transmission from infected to healthy individuals by the bite of a mosquito vector. As noted above (Section 2.2.1), disagreement with that was first expressed by Delaporte (1989). Later in two other books, Spielman and D'Antonio (2001) and Espinosa (2009) briefly expressed similar views. For clarity, the following description of Reed's contributions is divided into three parts: (1) Before Lazear's death, (2) After Lazear's death and (3) Appraisal.

2.6.1. Before Lazear's death

The work that was agreed for the four members of the Commission during the first months of its operation is described in Section 2.4.2 (above). Reed, Carroll and Agramonte would investigate *Bacillus icteroides* as a possible infectious agent of yellow fever, while Lazear was permitted to pursue his interest in possible mosquito transmission. Reed himself was in Washington throughout most of that period, making brief visits to Cuba, and the progress of Lazear's experimental work was barely known to the other members of the Commission. On one of his brief visits to Cuba, Reed met Carter and was shown the draft of his article on the period of extrinsic incubation. He also met Durham and Myers who were on their way to Brazil (Section 2.5.1).

Insights can be gained into Reed's attitude to the concept of mosquito transmission from letters and other communications from that period. At the time of his arrival in Cuba in 1900, Reed seems to have been aware of the theory of mosquito transmission of yellow fever, but how far he accepted it is not clear. On 4 July 1900, Carter, Carroll and another individual met Reed. When Carter asked him "You know what Finlay says about it being conveyed by mosquitoes?", he replied "Don't tell me, I want to keep an entirely unbiased mind on the matter." But Carroll maintained that it was conveyed by bedding and clothing used by the sick, to which Reed commented "That is so." Carter pointed out that large amounts of bedding and clothing brought from Havana to New York, when there was yellow fever in Havana, had not infected those that had unpacked it.[8]

On 23 August, Lazear wrote to his wife "Reed and Carroll… are interested in the controversy with Sanarelli and they think of that all the time… The malaria work is my own and they make no suggestion."[19] On 24 September, Reed wrote to Carroll "Now, concerning the mosquito propagation of the parasite, I am intensely interested, but I cannot say that any of your cases, except, perhaps Dean's, prove anything."[20] This suggests that at that time Reed had not taken seriously the discussions with Carter in July.

Five days after William Dean had been diagnosed with yellow fever, Agramonte and Lazear sent a cablegram to Reed "…apprising him of the fact that the theory of transmission of yellow fever by mosquitoes, which at first was doubted so much… had indeed been confirmed" (Agramonte 1915). On 25 September, the day that Reed heard of Lazear's infection with yellow fever, he wrote

Table 1. Analysis of the contents of the notebook left by Jesse Lazear at his death on 25 September 1900, as it was when purchased by the New York Academy of Medicine in April 1932. Pages bearing handwriting are mixed with blank pages. The overall text is separated here into 'Blocks' of pages that are separated by four or more blank pages and by differences in handwriting. The nature of the handwritings led to Blocks 1 and 3 being ascribed to Jesse Lazear and Block 2 to Walter Reed, findings confirmed by detailed analyses of the text by Carol Lowbeer, a graphologist, who also examined leaves 291 and 292. The subject matters comprising the Blocks were recorded from the Serial numbers printed.

Initial blank pages: 1–6

Block 1, pages 7–100

Writer: Jesse Lazear

First page in Block 1 (with handwriting): page 7. Dated: 2 March 1900.

Last page in Block 1 (with handwriting): page 100. Dated: 13 September 1900.

Jesse Lazear died on 25 September 1900.

Total number of pages in Block 1 = 94. Pages with handwriting = 58; blank pages = 37.

Subject matter: Patients with typhoid from p. ?? Patients with yellow fever from p. ?? dated 23 May 1990, later including experimental yellow fever.

Intervening blank pages: 101–104

Block 2, pages 105–104

Subject matter: Observations on the subject were measured continuously on 8 days between 25 January 1901 and 1 February 1902.

Ascribed to Walter Reed.

First page in Block 2: page 105. Dated 8 December 1900.

Last page in Block 2: page 143. Dated 2 February 1901.

Total number of pages in Block 2 – 39. Pages with handwriting = 15; blank pages = 16.

Subject matter: Experimental yellow fever.

Intervening blank pages: 144–169

Block 3, pages 170–197

Writer: Jesse Lazear

First page in Block 3: page 170. Dated 19 February 1900.

Last page in Block 3: page 197, undated; page 195 is dated 7 August 1900.

Subject matter: ??Experimental or observational malaria.

Intervening blank pages: 198–240

Block 4, two leaves, their front pages numbered 291 and 292 (being '*endpapers*' bound to the back cover).

Writer: Edward Weatherwalks

Individual examined: Edward (?eather(?)alks, New Jersey.

Period: Examined continuously on the 8 days between 25 January 1901 and 1 February 1902.

to his friend Major J. R. Kean: "You know that from the beginning of our work, I have insisted upon the common-sense theory of an intermediate host, such as the mosquito, being the carrier of the parasite of y. fever, but have said that human experimentation alone could determine the question."[21]

2.6.2. After Lazear's death

Jesse Lazear died on 25 September 1900. Major J. R. Kean sent a telegram from Quemados to Lazear's wife Mabel in Beverley, Massachusetts, with the message "Dr Lazear died at 8 this evening. *Kean*". (It was dated as received on 26 September 1900).[22] Kean must have cabled Reed with the same news, because on 26 September, Reed wrote to Carroll saying that Kean had informed him of Lazear's death.[23] His letter expressed his sorrow, and included two other matters that reflected his thoughts. The first, "I got the General to cable yesterday about securing Lazear's notes which he wrote that he had taken in each case bitten by mosquitoes. Examine them carefully & keep all." The second, "If your observations are such as you and Lazear have intimated, we must publish a preliminary note as soon as it can be gotten ready." Reed returned to Cuba in early October 1900, arriving in Havana on 4 October. On 7 October, Reed wrote to Finlay: "I have taken the liberty of sending my driver for the copy of the *British Medical Journal* containing Durham's and Meyer's notes."[24]

Using information from Lazear's notebook and other recent sources, Reed prepared a talk which he delivered on 23 October 1900 at the Annual Meeting of the American Public Health Association held in Indianapolis, just under a month after Lazear's death. His presentation was published later that year (Reed et al. 1900). Agramonte (1915) recalled that after Lazear's death "…all the data were carefully collected from Lazear's records and those at the Military Hospital, a short paper was prepared which the major had the privilege to read at the meeting… held on October 24 in the city of Indianapolis" [in fact, he spoke on 23 October]. In this article, Reed made generous acknowledgement of the earlier contributions of Finlay and Carter, but Lazear was given scant mention. Carter's demonstration of an extrinsic incubation period of 9 to 16 days was taken as consistent with the involvement of an intermediate host such as a mosquito, and the time required for an infectious agent to pass from the stomach of a mosquito to its salivary glands.

Reed's direct involvement in the work started upon his return to Cuba for two weeks after Lazear's death, when he initiated a program of work, under near field conditions, to substantiate Carter's demonstration of an extrinsic incubation period and Lazear's finding of transmission by *Culex fasciatus*. Reed returned to Cuba again in early November 1900. He agreed with Agramonte's choice of a site for an experimental camp, and a station named Camp Lazear was set up in an open field about one mile from the town of Quemados. Established on 20 November 1900, from that date the Camp was strictly quarantined. Tents were provided for the accommodation of volunteers, and mosquito-proof wooden buildings were constructed for the experimental work (Agramonte 1915). Personnel who conducted the trials were Dr. Roger P. Ames and Dr. R. P. Cooke, both Acting Assistant-Surgeons, a hospital steward and nine privates from the Hospital Corps (Reed et al. 1901a). Immune volunteers exposed to infection were referred to by name and case number.

Experiments to test transmission through fomites and by mosquito bite were undertaken simultaneously. Transmission through fomites was tested in a secure, small-frame building, which had been packed with the bedding of yellow fever patients that was heavily soiled with urine, faecal matter and the characteristic black vomit. During three periods of 20 days, the building was occupied at night by two or three non-immunes, who slept in the contaminated bedding, and in one case in the nightshirts of the patients. None of the volunteers became infected (Reed et al. 1901a, 1901b).

Subcutaneous inoculation of four non-immune volunteers with venous blood from yellow fever patients resulted in the infection of three, showing that the infectious agent had been present in the donors' circulations during at least the first to third (perceived) days of the disease, and that it could be transmitted in this way (Reed et al. 1901a). Further experiments confirmed these findings (Reed et al. 1901b, Reed 1902). Experimental transmissions were undertaken at Camp Lazear (Section 2.6.2) with the methods used by Finlay and Lazear.

One afternoon in July 1924, Carter talked to two friends, Drs. Thayer and Parker, about past events, and more openly than usual. His daughter, Laura Armistead Carter, took stenographic notes discretely during the conversation, and in 1925 sent a transcription of those notes[8] to a friend.[25] Carter recalled that the first attempts at experimental transmissions had been unsuccessful because, after feeding on a yellow fever patient, mosquitoes were kept only three days before biting a well man. "I told Reed that if my observations meant anything; the mosquitoes should be kept longer before the second biting since the period of extrinsic incubation had been fixed by my self as somewhat in excess of 10 days. This was done and justified by the results." From the context, it appears that Carter was referring to experiments at Camp Lazear, not to the earlier experiments by Lazear. If so, the cases, if any, with short periods of extrinsic incubation were omitted from the report published later by Reed et al. (1901a).

In their article, Reed et al. (1901a) detailed six attempts at experimental infection, to non-immune volunteers who had been bitten by small numbers of *Culex fasciatus* females that had fed on moderate, severe or fatal cases of yellow fever between 14 and 24 days earlier. One volunteer, Private Kissinger, who had been bitten 15 days after the presumed infection of one mosquito, remained uninfected. The other five, who had been bitten by mosquitoes infected between 14 and 24 days earlier, all came down with yellow fever. It was surmized that in the field the extrinsic incubation period would be about 12 days in summer weather and 18 or more days during the cooler winter months (Reed et al. 1901a). Further experiments revealed that mosquitoes could transmit the infectious agent 39, 51 and 57 days after becoming infected (Reed et al. 1901b).

The following conclusions were reached from the experimental studies at Camp Lazear. (1) Yellow fever is not transmitted by fomites. (2) It is transmitted through the bites of mosquitoes that have previously imbibed blood from persons sick with the disease (confirming Lazear's finding). (3) The mosquito *Culex fasciatus* serves as the intermediate host. (4) Females that have fed on an infected individual and that bite healthy individuals 2–10 days later do not transmit the infectious agent. An interval of about 12 days or more after its infection is needed before a mosquito can convey the infectious agent (confirming Carter's observations of the period of extrinsic incubation) (Reed et al. 1901b).

During the earliest years of investigations into the infectious agent of yellow fever, the existence of viruses could not be known because of the lack of filters impervious to bacteria. In 1884, the Chamberland filter, an unglazed porcelain bar with pores smaller than bacteria was developed. In 1892, material from infected tobacco plants that had passed through a Chamberland filter proved infectious. A similar finding in 1898 led to the re-introduction of the Latin word 'virus', and that infectious agent is now named tobacco mosaic virus. In 1898, the passage of an animal pathogen through a Chamberland filter was reported; it is now named foot-and-mouth disease virus. In Cuba, serum from a yellow-fever case was diluted and passed through a Berkefeld filter (of diatomaceous earth and impervious to bacteria). When inoculated into a non-immune individual it promptly induced an attack of yellow fever. The word 'virus' was not used for this case, but because it might be designated as ultra-microscopic, the infectious agent of yellow fever was compared with that of foot-and-mouth disease of cattle (Reed 1902).

On 22 November 1902, at Columbia Barracks, Walter Reed died from peritonitis, aged 51.

2.6.3. Appraisal

From 1900 through most of the twentieth century most reviewers credited Walter Reed alone with the discovery that the infectious agent of yellow fever is mosquito borne, holding him in great esteem. It was challenged by Delaporte (1989), and later by Spielman and D'Antonio (2001) and Espinosa (2009).

One reason for misconceptions of the extent of Reed's input could be that he was the senior author of all relevant publications; most notably of the first (Reed, Carroll, Agramonte and Lazear 1900), in which Jesse Lazear, who had undertaken all of the experimental work with mosquitoes and achieved the initial positive result, was mentioned only as a member of the Commission. There was no mention of him in the descriptions of the experiments which he alone had conducted that indicated that a mosquito acts as the intermediate host. Repeated use of the words 'we', 'our' and 'us' in the article, as in "The experiments made by us on eleven non-immune individuals …", give the impression of Reed's being closely involved at all stages, whereas in these experiments he certainly was not. The practice of scarcely mentioning others actively involved was followed in later articles (Reed et al. 1901a, 1901b, Reed 1902).

Carroll recognized this, and in 1906 wrote to Dr. Howard A. Kelly concerning statements in the manuscript of Kelly's forthcoming book *Walter Reed and Yellow Fever*. He wrote "We have a demonstration of the mosquito theory and confirmation of it, all the work of Lazear during Reed's absence in the United States." "Is it fair, is it just then to single out Reed and give to him the sole credit of a discovery that was made while he was more than a thousand miles away?"[9]

A partial explanation for Reed's practice can be found in Agramonte's (1915) account of the first meeting of the Board on 25 June 1900, when "It was unanimously agreed that whatever the result of our investigation should turn out to be, it was to be considered as the work of the Board as a body, and never as the outcome of any individual effort." Viewed in the context of the management of that meeting, it is pertinent that Reed was much older than and greatly superior in rank to the other three, who "…with a feeling akin to reverence heard the instructions which Major Reed had brought us from the surgeon general."

In contrast to the compliments to Finlay expressed in the initial article (Reed et al. 1900), it appears that Reed later expressed a different view in a letter to Major William C. Gorgas (Chief Sanitary Officer of Havana). Reed had been annoyed by Gorgas's acceptance of Finlay's theoretical contributions, and had bluntly criticized him for that. In responding to Reed's letter, Gorgas wrote "I do not 'honey buggle the simpering old idiot' a bit. I think he is an old trump as modest as he is kindly & true."[26] Much later, J. R. Kean described Finlay as a kindly and learned old man,[27] and Laura Armistead Carter wrote "No one was more keenly desirous that Finlay be accorded [the] honor justly due to him than Father. He both loved and revered the Great Cuban."[25]

To conclude, Walter Reed's contributions started when he, with Carroll and Agramonte, disproved the hypothesis that *Bacillus icteroides* was the infectious agent of yellow fever. They continued when, after Lazear's death, Reed returned to Havana from Washington and organized a programme of work to confirm Lazear's finding. By intensive exposure of volunteers to heavily soiled bedding and bed clothes, the theory of transmission by fomites was disproved. Despite Reed's earlier meetings with Carter, and his discussion on the etiology of yellow fever with Durham and Myers, it appears that the attempts at experimental transmission at Camp Lazear succeeded only after Carter had emphasized to Reed the importance of an experimental period of extrinsic incubation greater than ten days.[8]

3. Ensuing events in the twentieth and twenty-first centuries

An historical account of the discovery of the mode of transmission of a disease as important as yellow fever may reasonably extend beyond a description of the discovery itself.

3.1. Benefits from the advancement in knowledge

Major William C. Gorgas, Chief Sanitary Officer of Havana, put the knowledge that yellow fever was transmitted by mosquitoes into practical effect when he authorized the isolation of yellow fever patients in fully screened buildings, and employed brigades of soldiers to patrol the city, attacking mosquitoes during their aquatic and adult stages. After five months there were no further cases of yellow fever in Havana (Spielman and D'Antonio 2001). In fact, the older medical officers in Cuba were familiar with means of combating mosquitoes which they had learned from publications of the entomologist L. O. Howard, and Gorgas applied the methods that had been used in the neighboring town of Marianao since the year before.[28]

In 1904, when the United States attempted to complete the construction of the Panama Canal, Gorgas was appointed by the army to service in the Canal Zone. There he was obliged to work against the dislike and inclinations of the serving army officers and men, and also of civilians, but even so he achieved eradication of yellow fever. Malaria lingered but at a much lower level than before (Spielman and D'Antonio 2001).

Recognition of the infectious agent of yellow fever as a virus triggered new attempts to develop a vaccine. Isolation of yellow fever virus in West Africa in 1927 led to the development of two vaccines in the 1930s. In South America, through the use of vector control and strict vaccination programs, the urban cycle of yellow fever was, for a time, nearly eradicated. Today, yellow fever is controlled where vaccination is enforced.

3.2. Who deserved the credit?

Following centuries with heavy death rates from spasmodic epidemics of yellow fever in Cuba, and after the major epidemics in the United States during the nineteenth century, there must have been a strong inducement in both countries for individuals to claim the glory for the scientific advances that had made control of the disease possible.

In Cuba, Finlay was highly esteemed for his observational, theoretical and experimental contributions. With a growing sense of nationalism, informed individuals refused to acquiesce with the disregard elsewhere of Finlay's achievements. Espinosa (2009) described the anger that built up in medical circles and among public health officials, which persisted for decades. In a presentation to the Congreso de la Prensa Médica Latina held in 1927, Jorge Le-Roy lambasted American journals that gave all credit to Reed and the Commission, despite corrections published by Cuban sources (Le-Roy 1927). In the United States, through most of the twentieth century, Walter Reed was widely credited with establishing the mode of transmission of yellow fever, in disregard of the contributions of Finlay, Lazear and Carter. Throughout that period, reviewers failed to seek out all of the relevant published work, notably that of Finlay and Carter, and did not critically analyse the articles they had to hand.

Concerning reviews published later in the twentieth century, the comments of Chernin (1983) on reviews and biographies of the life of the parasitologist Patrick Manson are enlightening. He observed that: "eminent scientist-physicians who wrote these papers copied each other's mistakes and did not consult the original literature." Of the biographies he wrote: "historical records, once warped, resist adjustment."

NOTES

(1) Passive transfer of maternal immunity. In mammals, particles of an arbovirus that have infected a host are recognized as antigens and induce the production of antibodies, mostly glycoproteins of the immunoglobulin (Ig) family and principally of the classes IgG and IgM, which bind specifically with a corresponding antigen (Clements, 2012: Sections 44.2.3 and 45.6.5, d). IgG antibodies are among the few substances of high molecular weight that can traverse, to a significant degree, the histological barrier that separates the blood in the maternal and foetal circulations. This occurs through specific transport of IgG by the neonatal Fc receptor FcRn, which binds IgG at acidic pH and releases it at neutral pH (Palmeira et al. 2012).

(2) Finlay's hypothesis presupposed 'mechanical transmission'. This is a mode of transmission by which particles of an infectious agent that adhere to the mouthparts of a haematophagous arthropod that feeds on an infected host are transferred to an uninfected host if it feeds again immediately or within a few hours. Proof of mechanical transmission in the field has been obtained for pox viruses but not for any arbovirus. In fact, yellow fever virus is transmitted by 'biological transmission', a mode of transmission in which haematophagous arthropods are both a host and vector. An infectious agent that is ingested when a haematophagous arthropod feeds on an infected vertebrate invades the arthropod, replicates in certain of its tissues and accumulates within its salivary glands. When the arthropod feeds again at an appropriate time later, the infectious agent is transported in its saliva, and invades and infects the vertebrate host. Knowledge of the 'extrinsic incubation period', the mean time between ingestion of an infected blood meal and attainment of competence to transmit the pathogen by bite, is essential for epidemiological analysis of cases of biological transmission.

(3) Letter, Henry Rose Carter to Laura Eugenia Hook Carter, 14 July 1900. Hench-Reed Collection (see Note 4).

(4) The 'Philip S. Hench Walter Reed Yellow Fever Collection' is an archive of letters and other communications between American Army personnel involved in yellow fever investigations in Cuba, their military associates and their families, during the period of activity in Cuba and for many years before and afterwards. Hench, a physician, was co-winner with two others of the 1950 Nobel Prize for Physiology or Medicine, for the discovery of cortisone and its medical use. From about 1940 he became interested in the history of yellow fever research, and between then and his death in 1965 he assembled a collection of letters and other items concerning American activities in Cuba, which amounted to more than 7,000 items. Every item has been digitized for computer use and is searchable. The Collection is held in The Claude Moore Health Sciences Library, University of Virginia, and an on-line source is http://etext.lib.virginia.edu/healthsci/reed/guide.html/. Another collection of related items is the 'Reed/Carroll Letters', held in the Health Sciences and Human Services Library, University of Maryland. These letters describe activities of the Yellow Fever Commission during the period when Reed was in the United States. Its items have not been digitized.

(5) Conversation between Drs. Carter, Thayer and Parker, 1924, Henry Rose Carter Papers, Claude Moore Health Sciences Laboratory, Department of Historical Collections and Services, Box 1.

(6) Letter, George Miller Sternberg to Walter Reed, 29 May 1900. Hench-Reed Collection.

(7) Report: Statement regarding the work carried out by the Army Board, by Aristides Agramonte, 31 August 1908. Hench-Reed Collection.

(8) Article, by Laura Armistead Carter, July 1924 [Enclosed in C0312017]. Hench-Reed Collection. Original MSS said to be in the Library of the Surgeon General, later renamed the Army Medical Library.

(9) Letter, James Carroll to Howard A. Kelly, 23 June 1906. Hench-Reed Collection.

(10) Letter fragment, Jesse W. Lazear to Mabel H. Lazear, 15 July 1900. Hench-Reed Collection.

(11) Letter, Henry Rose Carter to Albert E. Truby, 4 March 1922. Hench-Reed Collection.

(12) Notes on Philip Showalter Hench's speech entitled 'Walter Reed and the Conquest of Yellow Fever', [19--]. In the University of Virginia Library. Virgo Feedback, Philip S. Hench Walter Reed Yellow Fever Collection. https://search.lib.virginia.edu/catalog/uva-lib:2229585/tei.

(13) Notes on Reed's laboratory notebook, New York Academy of Medicine, [19--]. Hench-Reed Collection.

(14) Letter, Laura Wood to Philip Showalter Hench, 24 November 1941. Hench-Reed Collection.

(15) Photocopy in the New York Academy of Medicine Library, Historical Collections, in a folder catalogued as 'New York Academy of Medicine. Historical Archives. Correspondence, 1917-1974, Reed, Walter. 1941-1942. Correspondence concerning the Reed notebook'.

(16) 'leaf' (pl. 'leaves'), a single piece of paper composed of two 'pages' back to back, a front 'page' and a back 'page'. In page numbering, front pages usually have uneven numbers 1, 3, 5, 7 and so on, while back pages have even numbers.

(17) e*ndpapers*. Classically, hardback books have two *endpapers*, which are so-named through being pasted one to its front hard cover and the other to its back hard cover. In detail, an *endpaper* is a large paper sheet that, folded once, give it the form of two A4-sized leaves, each with front and back pages. In a hard-back book, one half of an *endpaper* is pasted to the inside of the front board cover of the book, while its other half is pasted to the front side of the first leaf of the book. At the back of the book, a partner *endpaper* is pasted to the outside of the final page and to the inside of the board cover. In this way the two hard boards are securely bound to the stack.

(18) Personal communication from Philippa Colvin dated 21 May 2013, with scanned copies of pages in the notebook.

(19) Letter, Jesse W. Lazear to Mabel Lazear, 23 August 1900. New York Academy of Medicine Library, Historical Collections.

(20) Letter, Walter Reed to James Carroll, 24 September 1900. Hench-Reed Collection.

(21) Letter, Walter Reed to Jefferson Randolph Kean, 25 September 1900. Hench-Reed Collection.

(22) Telegram, Jefferson Randolph Kean to Mabel H. Lazear; received 26 September 1900. Hench-Reed Collection.

(23) Letter, Walter Reed to James Carroll, 26 September 1900; Health Sciences and Human Services Library, University of Maryland, Baltimore.

(24) Letter, Walter Reed to Cárlos Finlay, 7 October 1900; *Obras Completas* 6: 109, La Habana: Editorial Científico-Técnica, 1981.

(25) Letter, Laura Armistead Carter to Hugh S. Cumming, 15 October 1925. Hench-Reed Collection.

(26) Letter, William Crawford Gorgas to Walter Reed, 6 February 1902. Hench-Reed Collection.

(27) Letter, Jefferson Randolph Kean to Harry Clemons, 22 September 1939. Hench-Reed Collection.

(28) Letter, Jefferson Randolph Keen to Harry Clemons, 28 November 1939. Hench-Reed Collection.

AUTHORS' CONTRIBUTIONS

The bulk of this composition was compiled by the first author, Alan Clements, based on extensive examination and study of published articles, personal letters and other documents. It was originally prepared as an addendum (one of two addenda) to be included in a fourth volume of *The Biology of Mosquitoes* (Clements 1992, 1999, 2012), which unfortunately will not be completed. I, the second author, reviewed the addendum in 2015, and Alan incorporated corrections and changes into the manuscript based on my comments. I spoke with Alan in February 2016 and since further work on Volume 4 was unlikely, I encouraged him to publish the two addenda as review articles. Alan was to meet with me in the Natural History Museum in April 2016, but he was not able to travel to London due to poor health and advancing infirmity. I sent an email to him in December and again encouraged him to publish the addenda, and offered my assistance on that occasion. Alan finally replied to my email by telephone in June 2017. He asked me to prepare the addenda for publication and I happily agreed, without realizing the amount of time and effort that it would take. In a nutshell, my contributions include the following: a thorough review the manuscript; checking and verifying certain details and making corrections as required; editing and modifying the text for consistency; writing several small parts (abstract and acknowledgements as well as this paragraph); addition/revision of several sentences in the text; conversion of numerous footnotes, some duplicated between sections, into a single list of Notes; using photocopies of Table 1 and Figure 1 to prepare Word and jpg files, respectively; and reformatting the original document for publication as a journal article. This, however, palls in significance in comparison with Alan's brilliant investigative and creative efforts that produced this essay. I am honored to be listed as his co-author.

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