The study provided the following observations: (1) *Aedes aegypti* mosquitoes transferred the disease from an infected individual to a nonimmune person; (2) at least 12 days were needed for the extrinsic incubation period in the mosquito before it could transmit the infection; (3) yellow fever can be transferred to a nonimmune person from the blood of an infected individual taken during the first 2 days of the illness; (4) a filterable agent was responsible for infection; (5) the incubation period for humans ranged between 2 and 6 days; and (6) yellow fever cannot be transmitted by fomites nor spread in a house without the presence of mosquitoes. The most significant conclusion was that the “spread of yellow fever can be most effectually controlled by measures directed to the destruction of mosquitoes.”

See www.jama.com for full text of the original *JAMA* article.

**Commentary by J. Erin Staples, MD, PhD, and Thomas P. Monath, MD**

**Yellow Fever: 100 Years of Discovery**

_**SUMMARY OF THE ORIGINAL ARTICLE**_

_The Etiology of Yellow Fever: An Additional Note_  
Walter Reed, MD, Jas. Carroll, MD, and Aristides Agramonte, MD  
_JAMA. 1901;36(8):431-440_

The article describes a series of experiments conducted to explore how yellow fever is propagated from individual to individual and how the contagium is spread within households. The study was conducted in an experimental sanitary station in Cuba, where exposures and movements could be completely controlled. During the investigation, 12 nonimmune persons underwent different exposures, including mosquitoes that had fed on yellow fever patients, blood from infected patients, and fomites belonging to infected patients.

At the end of the 19th century, the United States invaded Cuba during its war with Spain. For every soldier who died in battle, 13 died of yellow fever. Surgeon General George Sternberg sent Walter Reed, Aristides Agramonte, James Carroll, and Jesse Lazear to Cuba to investigate the cause of yellow fever. Reed’s work proved that *Aedes aegypti* mosquitoes were the primary mode of transmission for the disease and that yellow fever was caused by a filterable agent found in the blood of infected patients.

Reed’s work led General William Gorgas to institute a campaign in Havana against the urban mosquito vector, eliminating the disease in 1902. He accomplished the same task 4 years later in Panama, which allowed the canal to be completed. By 1918, the Rockefeller Foundation formed a Yellow Fever Commission with the ultimate goal of eradicating yellow fever through the elimination of *Aedes aegypti*. While these early activities were initially successful against urban (*Aedes aegypti*–borne) yellow fever, the goal of eradication was dispelled with the discovery that yellow fever was...
a zoonosis, maintained by sylvatic mosquito species and non-
human primates in the Amazon jungle.3

It took more than a quarter of a century after Reed’s ob-
servation that yellow fever was caused by a filterable agent
before the isolation of the causative virus. In 1927, Adrian
Stokes isolated the virus from a sick man in Ghana known
as Asibi.3 In 1930, Max Theiler was able to identify a more
convenient animal model by demonstrating that mice were
susceptible to intracerebral inoculation of the virus.3 This
led to the development of a test for neutralizing antibodies
that was widely deployed for epidemiological and diagnos-
tic studies.

Theiler and colleagues passaged the Asibi virus more than
200 times in cell cultures. Testing of this subculture, desig-
nated 17D, revealed that the virus had become attenu-
ated but could still induce a protective immune response
in monkeys and humans.3 The 17D strain would serve as
the basis of the vaccine still in use today and is responsible
for saving untold numbers of lives. For this work, Theiler
was awarded the Nobel Prize in 1951. At about the same
time a second live attenuated vaccine was developed from
a different strain isolated in Dakar in 1927. This virus was
attenuated by serial passages in mouse brain yielding the
French neurotropic vaccine (FNV). This vaccine was widely
used through the 1960s in francophone Africa, leading to
the virtual disappearance of the disease.6

These early discoveries served as the foundation of sub-
sequent investigations of yellow fever over the ensuing de-
cades. Advances were made in the epidemiology, ecology,
diagnostics, etiology, and prevention of yellow fever. The
test for neutralizing antibodies developed by Theiler al-
lowed for the delineation of the geographic boundaries
of virus transmission. Tropical regions of both Africa and
the Americas were found to have endemic virus transmission,
but yellow fever was notably absent from Asia.

Ecologic assessments in Africa identified additional syl-
vatic mosquitoes capable of transmitting the disease.7,8 Ver-
tical transmission of the virus in mosquitoes, first reported
in 1905, was confirmed in the 1970s, explaining the long-
term persistence of the virus in nature.8 Considerable field
research efforts failed to uncover a role for animals other
than nonhuman primates in the circulation of yellow fever
virus.3

By 1960, hemagglutination-inhibition and complement
fixation tests were available, simplifying the detection of an-	ibodies to yellow fever virus.5 These tests also revealed that
yellow fever virus was antigenically related to other group
B arboviruses (now called Flaviviruses, named after the pro-
totype yellow fever virus, flava meaning yellow in Latin).
In the 1970s, viral detection was improved with the devel-
opment of insect cell cultures and inoculation techniques
for mosquitoes. Eventually enzyme-linked immunosor-
bent assay and reverse transcription–polymerase chain re-
action were developed, which further simplified and im-
proved antibody and viral detection.10 Serological studies

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ver occurs and other regions (along the coast) where yellow fever is nonendemic. Vaccination is practiced in the endemic but not in the coastal zones. Prior to the 1970s, the urban mosquito vector (Aedes aegypti) had been eradicated from South America, freeing these coastal regions from the threat of introduction and spread of yellow fever from the jungle to the urban transmission cycle. However, with the senescence of mosquito control and the huge expansion of cities along the coast, these regions are increasingly at risk. The ease of international travel also makes the introduction and spread of yellow fever into new areas infested with competent Aedes vectors possible, theoretically placing parts of Asia, Australia, Europe, and North America at risk.14 While outbreaks in many developed areas are likely to be identified and controlled quickly, they would significantly impact public health infrastructure and the medical system as well as tax the limited supply of 17D vaccine.

A major gap in the knowledge about yellow fever is how to manage and treat patients with this disease and with serious vaccine-associated adverse events. Treatment of yellow fever by supportive care is essentially ineffective, and improvements in intensive care have not changed the 50% lethality rate. Efforts to develop antiviral drugs for other flavivirus diseases, such as hepatitis C and dengue, should be expanded to include yellow fever. Rapid, point-of-care diagnostic tests not yet developed will be critical when an antiviral is available. In addition, current serologic tests are unable to differentiate between cross-reactive flaviviral antibodies, thus limiting the utility of the tests to definitively diagnosis the disease.

The complex interactions between yellow fever virus, cellular factors that control replication, and the innate and adaptive immune systems are poorly understood. Current work by basic immunologists is focusing on some of these factors, which may be key to understanding why only 1 of 7 people become ill after natural infection and why 1 in 200 000 people develop life-threatening viscerotropic adverse events to 17D vaccine that resemble wild-type yellow fever disease.10 Just recently, a cluster of viscerotropic adverse event cases occurred in Peru at a previously unprecedented incidence. Despite an intensive investigation, however, the cause remains elusive.15

Many questions remain about yellow fever disease, the etiological agent, and even about its natural transmission cycle. The threat of urban outbreaks and long-distance introductions is increasing. It is not understood why upswings in enzootic transmission occur and yet virtually no field research is currently being performed in this area. The 17D vaccine has been associated only recently with fatal adverse events, the pathogenesis of which are poorly known and improvements to the vaccine’s safety will be required. There are no specific drugs to treat the disease or severe reactions to the vaccine. While a significant amount has been learned in the last 100 years, clearly there is still much more to be discovered about yellow fever.

**Financial Disclosures:** Dr Monath is a director of Xcellerex Inc, a privately held company developing a new yellow fever vaccine. Dr Staples reported no disclosures.

**REFERENCES**