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Febre amarela, teorias biomédicas e práticas sanitárias: uma história de muitas faces

(texto inédito que traduzirei para português)

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This past July, Brazilian papers announced news in the fight against *Aedes aegypti*. Juazeiro, in Bahia, Brazil, became the first place in the Americas to serve as a laboratory for an experiment: the release of genetically modified mosquitoes that, after breeding with wild females, transmit a defect to their offspring, which then die in the larva stage.

Today *Aedes aegypti* is the transmitter of dengue fever in the Americas, but in the past it was the great villain of yellow fever (and in some places remains so).

Although there have been yellow fever outbreaks from the outset of European colonization, it was only in the late eighteenth and especially nineteenth centuries that yellow fever became the scourge of the continent. Along with bubonic plague and cholera, it formed the sovereign triad of international health (Jorge, 1930b, p. 7) as a consequence of the globalization of capitalism, which spurred remarkable growth in the circulation of goods, troops, and migrants around the world. Yellow fever raged from New York to Buenos Aires, on both the Atlantic and Pacific coasts, turning two cities – Havana and Rio de Janeiro – into "infectious volcanoes."

For most residents back then, yellow fever was a manifestation of divine wrath to be placated with prayers and processions. The contrast between the high death rate for whites, especially newly arrived Europeans, and the resistance of blacks led many masters to view the disease as a weapon of slave sedition.

The physicians that then began addressing public hygiene blamed the environment for yellow fever, citing both the "nature" of the torrid latitudes, considered hostile to European acclimatization, and the artificial environment created by urban man. Epidemics "blossomed" as regularly as seasonal fruit in coastal lowlands, and especially

in port cities filled with rotting matter, always emerging during the hot, rainy period, whose length varied with latitude.

To restore the balance of these urban "organisms," drastic interventions of a greater or lesser degree were proposed. Swamps were considered foci of miasmas. Hills and narrow streets blocked the wind circulation that might have dissipated them. The dwellings of the poor released clouds of pestiferous gases. Bodies buried inside churches, dead animals thrown into the streets, raw sewage in open ditches, slaughterhouses, hospitals, and prisons were just as guilty of soiling the air.

In the second half of the nineteenth century, the second Industrial Revolution – the revolution of iron and steel, of steam trains and ships – consecrated England as a world power while having other consequences in the Americas, which witnessed the abolition of the slave trade, the consolidation of nation-states, the advance of agro-export economies, the growth and modernization of port cities, and increased migration to these ports. This aggravated the sanitary situation in many of these cities, where yellow fever was considered a most serious problem precisely because it struck immigrants harder. Amidst these violent epidemics, a new actor made its debut in explanations of urban insalubrity: the microbe.

In December 1879, Dr. Domingos José Freire, of the Rio de Janeiro School of Medicine (Faculdade de Medicina do Rio de Janeiro), announced his discovery of *Cryptococcus xanthogenicus*; soon he had developed a vaccine against yellow fever that was to be used not only in Brazil but in Puerto Rico, Jamaica, and the Guianas (Benchimol, 1999, pp. 119-68). In Mexico, Manoel Carmona y Valle (1885; 1882, pp. 411-22) developed another vaccine with *Peronospora luteum*. Juan Carlos Finlay's *Micrococcus tetragenus* was conceived at the same time that he was using mosquitoes infected by yellow fever sufferers as live immunizing agents against the disease. In 1890, George Sternberg, chair of the Public Health Association, drew up a damning review of these and other theories and began looking for proof of a microbe resembling cholera's. Robert Koch, discoverer of the latter (1884), saw analogies with yellow fever, whose main symptom – black vomit – was likewise located in the intestine. In the 1890s, bacilli were also in the running as the causative agent of the disease.

Based on miasmic theory, the relative consensus about how to sanitize ports like Rio and Havana gave way to controversy that only grew more heated when there was another shift in the approach to yellow fever. Two events underpinned this reversal: Finlay's introduction of the theory of mosquito transmission, in 1880-81, and its proof twenty years later by the U.S. team headed by Walter Reed (1900) (Stepan, 1978, pp. 397-423; Delaporte, 1989).

The Americans only conceded defeat to Finlay after occupying Cuba in 1898 and having to face their patent inability to deal with yellow fever there (Stepan, 1978). Likewise important was the presence on the island of the British, who were investigating the rich question of the biological vectors of disease.

In 1898, Ronald Ross uncovered the cycle of the avian malaria parasite in *Culex*, and the following year, Giovanni Battista Grassi and his collaborators revealed the cycle of the human malaria parasite in *Anopheles*.

In June 1900, Herbert Edward Durham and Walter Myers, of the newly founded Liverpool School of Tropical Diseases, headed to the Amazon to investigate yellow fever. At a stopover in Havana, Durham and Myers had the chance to exchange ideas with Cuban doctors and members of the Walter Reed commission, and they came away with greater confidence in Finlay's hypothesis of yellow fever transmission via mosquitoes. In August, soon after they left Cuba, Lazear initiated experiments with mosquitoes supplied by Finlay, while Carrol and Agramonte advanced what were by then priority studies on the alleged yellow fever bacillus. In September, Lazear died of an accidental bite. Walter Reed hurriedly wrote a preliminary note and began a series of better controlled experiments to prove that the mosquito was the host of the yellow fever "parasite," that the disease was not transmitted by air, and that fomites were not contagious (Reed et al., 1900, pp. 37-55).

In 1901, the Reed commission presented its results before the Third Pan American Congress in Havana (Reed, Carroll, Agramonte and Lazear, 1901), while William Gorgas inaugurated the mosquito campaign there and Theobald finished the first volume of *A monograph of the Culiciidae or Mosquitoes*. When the latter established the genus *Stegomyia,* he included the *Culex* species associated with yellow fever, among them *Culex taeniatus*, identified by Adolpho Lutz in São Paulo, where

Reed's experiments were replicated in an effort to neutralize reactions to the so-called Havanese theory by physicians who still blamed transmission on bacilli and fungi (Benchimol and Sá, 2005, pp. 43-244; Cerqueira, 1954). The Reed commission's findings were also verified elsewhere; Rio de Janeiro, for example, was visited by researchers from Hamburg's Institute for Maritime and Tropical Diseases (Institut für Schiffs- und Tropenkrankheiten) and, for longer stays, from the Institut Pasteur in Paris.

The yellow fever campaigns driven by Oswaldo Cruz in Rio de Janeiro, by Gorgas in Havana and Panama, and by the British and French in spots in West Africa were grounded in the idea that the unknown yellow fever agent had only two hosts: humans and a single mosquito species – rechristened *Aedes aegypti* in the 1920s.

In 1905, two other researchers from the Liverpool School disembarked in Manaus to study yellow fever in the Amazon region. Both caught the disease. Anton Breinl returned to England but Harold Howard Shearme Wolferstan Thomas stayed on as head of the Yellow Fever Research Laboratory.

The Liverpool School (Power, 1999) had already sent expeditions to Africa, first targeting malaria and then trypanosomiases. It was in this arena that Thomas gained recognition, showing in 1903 that an arsenic compound, atoxyl, appeared to be effective in treating sleeping sickness and animal diseases caused by trypanosomes. Paul Ehrlich visited Thomas' laboratory, and in 1910 his own research with atoxyl led him to Salvarsan, the first effective syphilis drug (Riethmiller, 1999).

The laboratory in Manaus had ties with this cutting edge in medical research. The Reed commission had left unresolved the hypothesis that the yellow fever agent was a "filterable virus," although the term should not be construed as equivalent to our current notion of virus. Analogies drawn between malaria and yellow fever prompted many researchers to believe the agent was a protozoan, Finlay among them. In 1905, Fritz Richard Schaudinn and Erich Hoffmann announced the discovery of the syphilis agent, *Spirochaeta pallida* (*Treponema pallidum*). On the eve of this discovery (1904), Schaudinn reported on experiments with the *Athene noctua* owl, in which he had encountered forms whose life cycles could include mosquitoes as intermediate hosts. There, the *Spirochaetae* were so small they could pass through the finest bacteria filters. Schaudinn believed these might also be yellow fever agents (1904, pp. 566-73). This

was the hypothesis that Otto and Neumann, from the Hamburg school, investigated during their stay in Rio de Janeiro in 1904. Three years later, Arthur Marston Stimson, of the U.S. Public Health Service, found *Spirochaeta interrogans* in a yellow fever victim. This theory gained much ground during World War I, when the Japanese named *S. icterohaemorrhagiae* as agent of Weil's disease, or hemorrhagic jaundice, known today as leptospirosis.

The short circuit between Schaudinn's hypothesis and yellow fever was "closed" by Hideyo Noguchi, bacteriologist with the Rockefeller Institute, who in 1918, in Guayaquil, Ecuador, described a spirochete as agent of the disease. He established a new genus, *Leptospira,* which encompassed both Inada's agent and the agent of yellow fever, *Leptospira icteroides* (Cueto, 1994, 1995; Plesset, 1980; Benchimol et al., 2009).

The Rockefeller Foundation had decided to eradicate yellow fever using the "key center" theory. They would wipe out the places where *Stegomyia fasciata* could breed but only in a few endemic centers along the coast, from where the disease spread to inland settlements. When the campaign finally began in Guayaquil in November 1918, it seemed that science understood all essential aspects of yellow fever. By 1922, the Rockefeller Foundation's International Health Board stated that the east coast of South America was virtually free of the disease. The Brazilian government then decided to accept cooperation from the Rockefeller Foundation (May 1, 1923). An epidemic had exploded in Northeast Brazil, as historians can observe from Noguchi's laboratory in New York, for the actors engaged with the outbreak relied heavily on the sera and vaccine produced there.

In 1927, Noguchi came into the firing line of laboratories versed in the techniques of immunology. At the Harvard School of Tropical Medicine, for example, Max Theiler and Andrew Watson Sellards noted that the reactions of *L. icteroides* and *L. icterohemorrhagiae* were identical. These and other publications made it evident that there were gaps in a complex research program. If *L. icteroides* did not cause yellow fever, this meant cases of leptospirosis were being misdiagnosed, as Rockefeller staff in the Americas grounded themselves on Noguchi.

At the same time, some specialists believed infectious jaundice to be the yellow fever of temperate zones. These and other unsettled issues demanded more experiments

and, in the opinion of Theiler and Sellards, the idea that the cycle of the yellow fever agent was confined to man and mosquito had to be submitted to serious review.

Let us now shift our attention to West Africa. In June 1920, Gorgas accepted the assignment to lead a commission entrusted with ascertaining whether the measures adopted in the Americas would be viable in Africa. He died on his way there, in London (June 4), and his place was taken by Juan Guiteras Gener, a collaborator of Finlay's. Research focused on the coast from Lagos, capital of Nigeria, then a British colony, to Dakar, capital of the French colony of Senegal, both ports with large white populations, deemed more susceptible to yellow fever.

Studies on the two *Leptospira* and attempts to identify authentic clinical cases of yellow fever met with failure.

Ever since an enquiry by a British commission headed by Rupert Boyce in the 1910s, some had believed that a mild form of the disease was endemic among indigenous populations and from time to time would grow virulent for Europeans. Doctors back then firmly believed blacks were resistant to yellow fever. "Is there a natural race resistance?" Guiteras asked. The stories he heard in Africa and his own statistical inferences prompted him to draw a correlation between the low number of whites who caught it and the limited range of the disease, which he thought was dying out on that continent.

These unresolved issues had to do with a matter of historical nature. Yellow fever had supposedly originated in the Americas. Africa had never had hubs of the disease on the same scale as Havana or Rio de Janeiro. Upon disembarking in Santo Domingo, Columbus is said to have found the disease endemic among indigenous peoples of the Caribbean and Gulf of Mexico. Africa was thought to have been contaminated by slave ships, with the first epidemics breaking out in Senegal and the islands of Cape Verde in 1763. With yellow fever almost under control in the Americas, it would of course die out on the other side of the Atlantic.

In the early 1920s, Henry Rose Carter, member of the commission that had charted key centers in the Americas, began research on the origins of yellow fever, published in book form posthumously (1931). Backed by an arsenal of historical documentation, Carter endorsed the theory defended by Emilio Goeldi, former director of the Pará

Museum of Natural History and Ethnography, according to which *Stegomyia fasciata*, and therefore yellow fever, were African in origin. For Goeldi, the disease had migrated to the Americas on slave ships, like other human parasites – the chigoe flea (*Tunga penetrans*) and filariasis, for example.

In 1925, a second Rockefeller Foundation commission, headed by Henry Beeuwkes, was sent to Lagos. For two years they examined many cases but failed to isolate Noguchi's microorganism or to draw a clear epidemiological profile of the disease. This fueled suspicions that African yellow fever was different from its American counterpart. There was a marked contrast between the situations on each side of the Atlantic. If public health workers in Africa were spinning their wheels, in the Americas yellow fever seemed to be almost extinct. The director of the Rockefeller Foundation's International Sanitary Division, Frederick Russel, had authorized personnel in Brazil to begin shutting down operations and had transferred men to Africa.

Because of an outbreak of suspicious fevers on the Gold Coast, a branch lab was set up in Accra under Johannes Bauer, an assistant of Noguchi's. Prior to early 1927, the West Africa Yellow Fever Commission had not found any animal to be susceptible, not even the guinea pigs customarily used in tests with strains of *Leptospira icteroides* and *Leptospira icterohaemorrhagiae*. In May, Beeuwkes went to Hamburg and bought some rhesus and crown monkeys from India and saguis from Brazil. He then set off for Lagos with Adrian Stokes, member of the first commission sent to Africa and one of the first in Europe to verify Inada's discoveries.

In Kpeve, near Accra, they drew blood samples from patients with mild infections, one of whom was a 28-year-old African male named Asibi. Days later, monkeys and guinea pigs were inoculated with blood from groups of human cases, as it was hard to tell if any one individual had the disease. Some monkeys died, with changes suggestive of yellow fever. In a report dated July 14, 1927, the search narrowed: a rhesus (*Macaca mulata*) inoculated with material from Asibi displayed very eloquent signs. However, there were still many unresolved issues on both sides of the equation. There were few reliable tools for diagnosing human yellow fever, especially mild cases, and very little was known about incubation of the virus in monkeys, whose normal and pathological

histology was poorly understood. And how were they going to preserve the few strains they obtained? It was hard to come by Indian monkeys, subject to devastating illnesses. They could be infected by mosquitoes but a whole new slew of problems was raised by these microorganisms, invisible to the most powerful microscopes and only detectable in the lesions produced as they moved tirelessly from organism to organism.

The Americans and British who were collaborating with them needed a clear-cut human case produced by the virus. Human experimentation did indeed take place in Lagos but it was involuntary, and dramatic: Adrian Stokes was hospitalized on September 15, 1927 and died four days later, which had the effect of rapidly accelerating the pace of work.

The next year a preliminary note and then a more comprehensive article were published by Stokes, Bauer, and Hudson, showing that the infection was transmitted from monkey to monkey and from monkey to man, by blood injection or by *Aedes aegypti* bite. For his part, Bauer reported on the transmission of yellow fever by three other mosquito species (*Aedes luteocephalus*, *A. apicoannulatus*, and *Eretmapodites chrysogaster*).

In November 1927, Noguchi disembarked in Accra and started to work independently of researchers in Lagos. For a while, his observations were in tune with those of the rival laboratory, but eventually they took him in a totally different direction, which, if proven, would corroborate the idea that American and African yellow fever were in fact distinct diseases, kindred species like *Leptospira icteroides* and *icterohaemorrhagiae*. He was preparing his return to New York, with material to continue his studies there, when he was hospitalized. He passed away on May 21, 1928. During Noguchi's last days, William Alexander Young, director of the British hospital in Accra, did all he could to preserve evidence of Noguchi's work. Eight days later, he too died of yellow fever.

In this ill-fated month of May, in 1928, Rio de Janeiro saw the outbreak of an epidemic that demolished any hopes that it would be an easy task to eradicate yellow fever.

The newly discovered evidence from West Africa inspired a flurry of experimental studies and intense exchanges of information between Europe, the

Americas, and Africa. The virus – now a term with much the same meaning as today – was still shrouded in mystery. In the 1920s, yet another characteristic of these microorganisms, then linked to 64 other diseases, was identified: they depended upon living cells to reproduce (Hughes, 1977, pp. 93-108). New biochemical techniques for manipulating viruses were being developed in the field of virology, then breaking out of its Pasteurian cocoon.

Two lines of research were immediately opened: verifying the identity of the "African" and "American" viruses and determining whether other vertebrates or invertebrates were susceptible (Soper, 1937, p. 380; Aragão, 1929, p. 6; Aragão, Jul. 1929, p. 849).

The diagnosis of yellow fever meant interpreting often misleading clinical signs or relying on observations of lesions after death. To know whether someone had had the disease, their serum was injected into a monkey, which was then tested to see if it was protected against the infection caused by the virus. This technique could be used on a wide scale after 1930, owing to Max Theiler's discovery that when white mice received intracerebral inoculation, they died of encephalitis. Thanks to the new culture medium, new breeds of virus were obtained, with properties not observed in human and animal hosts.

Another retrospective diagnostic tool employed by hospital pathologists was converted into a technique applicable by non-specialized personnel in regions where dissecting corpses was a grievous sin. This was the viscerotome, an instrument with a handle and blade that could be used to remove a fragment of liver from people who had died with suspect fevers. Viscerotomy posts were set up around Brazil, while systematic study also began into the distribution of yellow fever immunity using mice, which provided the compass of a vast survey that revealed the problem to be much broader than imagined.

In 1930, Fred L. Soper instigated a total reorganization of Brazil's yellow fever service, taking advantage of new techniques for visualizing the disease and also of the Revolution of 1930, which ushered in a political setting more favorable to the vertical control of vectors and humans. With the intent of completely eradicating *Aedes aegypti*, all buildings were numbered and inspected and urban control areas were divided up so

that one inspector could cover each territory in one work week. Landlords were fined if they hampered efforts to combat yellow fever. At corners, arrows on signs told inspectors which direction to take. Inspection staff were now part of the Service's strict pyramid structure for supervising both the population and the work of their personnel (Soper et al., 1943, pp. 3-4; Lowy, 1998-9, p. 661).

In a 1933 publication, Fred Soper and his Brazilian collaborators reached the conclusion that in American forests the virus was also transmitted by vectors other than *Aedes aegypti* and that it had other vertebrate hosts besides people.

The findings of research on the distribution of yellow fever immunity in Northern, Southern, and Central America were presented in 1937 (Soper, Jul. 1937, pp. 457-511; Sawyer, Bauer and Whitman, Mar.1937, pp. 137-61). Three scenarios were depicted: an urban disease transmitted by *Aedes aegypti*; a rural disease, dependent on the same vector; and a disease bred in the vast, largely uninhabited jungle regions where sylvatic yellow fever attacked adults who ventured into the forest. Sometimes the latter was the source of urban epidemics but it could remain active without striking nearby cities even when the mosquito index there was high (Soper, 1939a, p. 5; 1939b, pp. 6-7).

In early 1938, the virus was isolated in monkeys bitten by *Haemagogus capricorni* and *Aedes leucocelaenus*, and also in mice subjected to *Sabethines* (Shannon, Whitman and França, 1938, pp. 110-1). In Colombia it was noted that mosquitoes were abundant in the crowns of trees (Soper, 1942, p. 5). New collection methodologies made it possible to identify other species associated with the transmission of yellow fever and other arboviruses (Consoli and Oliveira, 1994, pp.102-34). The epidemic that spread through Brazil in 1938 was marked by a high mortality rate among howler monkeys (genus *Alouata*).

Serological research on the African continent conducted by the Rockefeller Foundation and European institutions also permitted a more precise definition of yellow fever's endemic zone. In a broad belt from the western to eastern coasts and from the edge of the Sahara to Angola, between latitudes 15º north and 10º south, the disease manifests as sporadic sylvatic cases, outbreaks in rural areas, and, more rarely, urban outbreaks. In addition to *Aedes aegypti*, at least 16 species would prove capable of transmitting the yellow fever virus. The enzootic cycle in tropical forests, from monkey

to monkey, is sustained mainly by *Aedes africanus*. Human infections display an endemic pattern, with intermittent epidemics in humid savannahs during rainy seasons (Monath, 1991, pp. 31-5; Meegan, 1988, p. 219; Nasidi, 1988, pp. 41-2).

From 1933 on, the American continent witnessed no urban epidemic born of another and caused by *Aedes aegypti*. Urban flare-ups occurred when the virus was transferred from the forest to the city (1939, p. 7). The last episode of this nature had been reported in 1942, in Sena Madureira, in the territory of Acre, until urban yellow fever appeared in 2008 in San Lorenzo, Paraguay, an episode I will return to later.

Still, the extensive presence of sylvatic yellow fever – from Panama to Argentina and from Peru to Bahia – showed that the illness had adapted to a wide variety of ecologies (Soper, 1942, p. 2). Vaccination of people in contact with forests would be the only way to forestall urbanization of the sylvatic virus.

By 1940, the Yellow Fever Service, now run solely by Brazilians, had succeeded in eliminating *Aedes aegypti* over wide stretches of Brazil (Franco, 1969, p. 135), but the mosquito persevered at the far ends of uncontrolled streets, on farms, along roads and train tracks. The eradication program picked up pace in 1947 when DDT came into use. The so-called perifocal method was quickly adopted to simultaneously do away with the aquatic and winged forms of the insect. The campaign reached its apex in 1950, encompassing 112,950 locations and 3,249 staff members in Brazil.

[DRG1] Comentário: Jaime, caso voce já não conheá, experimente essa ferramenta genial: vai no google e entra: define: persevere - quando o resultado aparecer, clique no símbolozinho do lado direito (alto falante)

In 1942, besides Brazil, only Peru, Bolivia, and British Guiana were concerned with eradicating the mosquito. The Pan American Health Organization approved a plan of continental scope in October 1947, the year Soper became head of its executive agency, the Pan American Sanitary Bureau (PASB). Eleven years later (Oct. 2, 1958), the Fifteenth Pan American Sanitary Conference declared Brazil, the Canal Zone, and nine more countries (Belize, Bolivia, Ecuador, French Guiana, Nicaragua, Panama, Paraguay, Peru, and Uruguay) free of the urban vector (Franco, 1969, pp. 144-5). The species was not eliminated in the United States, part of Colombia, and six other countries (Suriname, Haiti, Venezuela, Jamaica, Dominican Republic, and Cuba).

As stated earlier, sylvatic yellow fever made vaccine development imperative. Starting in 1928, serum from convalescent patients was used to protect researchers working with the disease. That year, Theiler and Sellards showed that an injection with

serum and virus produced active immunity in monkeys (Theiler and Sellards, 1928, pp. 449-460; Hudson; Philip and Davis, 1929, pp. 223-232), but "serum-vaccination" was deemed risky after human testing in Rio de Janeiro and Bahia (Soper, 1937, p. 380).

In 1928, Edward Hindle (1929), in England, and Henrique Aragão (1928a, 1928b, 1929) and Lemos Monteiro (1929), at the Oswaldo Cruz and Butantã institutes, made vaccine from monkey livers and spleens, using chemical methods to attenuate virulence. Aragão's vaccine was administered to some 25,000 people in Rio de Janeiro.

An alternative to replication of the virus in live animals materialized in 1931 when the avian smallpox virus was cultured from the chorioallantoic membrane of chick embryos. It was later ascertained that several membranes of embryonated eggs were susceptible to infection by different viruses. We will soon see the importance of this.

Earlier I mentioned encephalitis in white mice inoculated intracerebrally. When the virus was "fixed" in this fashion, it behaved differently than the virus that provoked lesions in organs like the liver (Soper, 1937, p. 381). It was determined that after several passages through mouse brains, this "neurotropic" virus lost its ability to cause visceral lesions in monkeys, although it still attacked the central nervous system. In addition to affording a method of retrospective diagnosis, Theiler's discovery thus inaugurated a new stage in development of a yellow fever vaccine. In 1931, immunization experiments were conducted using the neurotropic virus combined with human immune serum (Sawyer, Kitchen, and Lloyd, 1931, 1932; Theiler and Whitman, Jul. 1935, pp. 1342-7; Soper, 1937, p. 382).

The New York lab was targeting two goals: modifying the virus by changing the conditions under which it was cultured, so that the resultant strain would display fewer adverse effects and greater immunizing power; and obtaining sera richer in antibodies – so-called hyperimmune sera – to better protect people from the risk of the vaccine itself (Soper, 1937, p. 383).

In 1936, Lloyd, Theiler, and Ricci successfully cultured a virus derived from Asibi, the African gentleman mentioned earlier, in embryonic mouse tissue. His blood had been inoculated into a rhesus monkey on June 30, 1927, and over the subsequent six and a half years, the virus had made passages through mosquitoes and other monkeys. The letters following experiment numbers indicated which culture media was being tested.

17E – the product of culturing *Asibi* in embryonic mouse tissue with normal monkey serum – was used in human vaccination with human immune serum.

Another route taken in 1934 yielded 17D, from the same origin but modified through successive cultures in different media, until arriving at in vitro passage through embryonic chick tissue from which the central nervous system had been removed. Using subculture 214 – counting from the original *Asibi* – many parallel series were begun, some in embryonated eggs. In 1937, the process yielded what Brazilians called a "friendly" virus, which protected rhesus monkeys in subsequent inoculations with virulent material and no longer occasioned encephalitis when injected into their brains (although it still did so in mice).

With 17E still in use, 200 people in Rio de Janeiro were vaccinated during February of 1937 (Soper, 1937, pp. 386-7). In March, a laboratory to produce the vaccine opened on the campus of the Oswaldo Cruz Institute. By the end of that year, in municipalities of Minas Gerais stricken by sylvatic yellow fever, 38,077 people were vaccinated, including men, women, and children over the age of two. Another 49,000 received coverage during a single week in January 1938. The vaccine was used in Colombia, where production itself began in January 1939 (Groot, 1999, p. 269; Soper, 1939a, pp. 13-4). In Asuncion, Paraguay, the 17D virus was used for the first time to combat an outbreak of urban yellow fever (Dec. 1937). In 1938 in Brazil, a wide-ranging sylvatic epidemic prompted the immunization of over one million people (1,059,328), primarily in rural settlements (Soper, 1939a, pp. 16-17).

The vaccine had to be kept at a low temperature, all the more complicated at a time when refrigerators were still rare. Extremely sensitive to direct light and heat, it was rehydrated and diluted just before inoculation. To make sure the virus had survived the trip, each vaccination unit had mice. When a session began, the first dose from each vial was used for intracerebral inoculation of a group of mice, while the last dose was used on a second group, both of which were observed for 21 days. Ending up with an inactive virus – that is, one with a low titer – wasn't the only risk: the wild virus of yellow fever would kill mice as of the third day, but if a mouse died before this, the problem was obviously more serious (Fonseca da Cunha, interview, tape 02).

The move from laboratory to large-scale vaccination was not seamless. At the Oswaldo Cruz Institute lab, Hugh Smith and Henrique de Azevedo Penna made important changes to the technique developed in New York to boost vaccine yield (Soper, 1939, p. 14; Lowy, 2000, p. 6). The virus was cultured in live chick embryo, in fertile eggs. On the fifth day, the embryos were extracted, minced with normal human serum, and filtered.

The laboratory managed to produce the doses needed for mass vaccination but its technicians had to resolve a series of grave problems. In 1939, certain vaccine batches achieved an immunization rate of only 20% (Bica, 1988, p. 164). The vaccine's decreased antigenic strength was blamed on the many transfers of the virus, which had surpassed 300 subcultures. The maximum number of subcultures was then set at 255 and the minimum at 210 (Soper, Smith and Penna, Sep. 1939, pp. 351-2).

Vaccination resumed but a new problem cropped up: so-called catarrhal jaundice (Lowy, 2000, pp. 4-7). The first cases had appeared when the 17E virus was in use (1936-37) and had been linked to hyperimmune animal sera (Findlay and MacCallum, 1937, 1938). These were abandoned when the switch was made to 17D, but normal human serum was still used, now to protect the virus itself. A study among those vaccinated in Espírito Santo in 1939-40 identified 1,000 cases and 22 deaths (Soper, 1942, pp. 8-9; Fox, Manso, Penna, and Pará, 1942, pp. 68-116). The technique was modified, eliminating the human serum suspected of transmitting a virus that only later was tied to hepatitis B.

In November 1940, vaccination resumed again with a new 17D strain brought from New York, but the next year a third serious problem surfaced: cases of encephalitis among the immunized, due to a mutation of the "friendly" virus itself (Fox, Lennette, Manso, and Aguiar, 1942, pp. 117-42). The seed lot system was then introduced, later adopted worldwide in the manufacture of other vaccines (Soper, 1942, p. 9).

The problems at the laboratory in Rio de Janeiro seemed to be under control but not at the New York lab, which was still using human serum. It had to move quickly to large-scale production, and this change in technique would require a number of tests. Output was around 56,000 doses in January 1941 and nearly 8 million (7,719,120) by December, shortly after the U.S. entered the war. Fearing a biological attack from

Japan, which had invested heavily in this type of weapon (Harris, 1994), in January 1942 the U.S. government decided to vaccinate its entire army (Furmanski, 1999, p. 822). That March, over 28,000 cases of jaundice were detected among newly vaccinated soldiers in California, resulting in 62 deaths (Soper, 1942, pp. 8-9; Lowy, 2000, pp. 10-15). Later research with veterans showed that about 330,000 of them had been infected. It was the largest hepatitis epidemic recorded in the annals of public health (Seeff, Beebe, Hoofnagle et al., 1987, pp. 965-70; Norman, Beebe, Hoofnagle, Seeff, 1993; pp. 790-7).

In the 1950s, the laboratory in Rio de Janeiro met the needs of Brazil, other South American countries, and, less regularly, of Africa, Europe, and Asia. Production surpassed ten million doses (1952 and 1953) as part of the drive to contain the new wave of sylvatic yellow fever that swept Brazil from north to south. From 1957 to 1959, while the elimination of *Aedes aegypti* in various parts of the continent was announced, the Paraguay and Parana river valleys and Central-Western Brazil saw outbreaks (Soper, 1977, pp. 1887-9). Several eruptions of sylvatic yellow fever occurred in the Americas in subsequent decades (1966).

In 1967, *Aedes aegypti* re-emerged in northern Brazil (Pará) and gradually regained its initial territory on the continent. A network of viscerotomy posts was re-established (1979) and a five-year vaccination cycle was implemented in regions exposed to sylvatic yellow fever (PAHO/OMS, 1980, p. 13). In 1980, 4.1 million doses were administered, rising to almost 18 million the next year. The goal was to reach people drawn to the Amazon and Central-Western Brazil by huge settlement, mining, and public works projects. The seriousness of this menace was underscored by the news that the actor Jason Robards had caught yellow fever while filming *Fitzcarraldo* with director Werner Herzog in the Amazon rainforest (*Veja*, Apr. 22, 1981).

Warning that the disease could re-urbanize, a number of organizations and specialists argued that generalized vaccination was less advantageous than vector eradication. A continental response to *Aedes aegypti* had to be re-engineered.

Outbreaks of sylvatic yellow fever in the interior of Brazil and foci of *Aedes aegypti* along the coast collided with each other in Roraima in 1982. The crash produced not the feared urban yellow fever but Brazil's first dengue outbreak. The latter

malady had emerged as a complementary threat shortly before, following the Cuban epidemics (1977 and 1981) (Martinez et al., 1987, pp. 148-57). The infestation of Rio de Janeiro brought about another dengue epidemic in 1986-87, caused by the type 1 flavivirus. *Aedes albopictus* was then detected for the first time (May 1986). Originating in Asia, where it transmits dengue fever and Japanese encephalitis, this mosquito apparently entered Brazil via ports that were exporting iron to Japan (Consoli and Oliveira, 1994, pp. 118-9).

Waves of yellow fever in the Afro-American belt and fear of an epidemic outbreak in uninfected areas of the Far East (OPAS/OMS, Feb. 1984) led to the organization of an international symposium, held in Belém in April 1980, to review aspects of the disease in the light of virology, molecular biology, and genetics, now equipped with more sophisticated tools.

Notable progress came in the production and dissemination of vaccines (Moulin, 1996) following the 1974 creation of the Expanded Immunization Program, a WHO initiative, and the 1977 creation of PAHO's Revolving Fund, which revolutionized the vaccine market. The Rio de Janeiro Laboratory, newly part of Bio-Manguinhos at the Oswaldo Cruz Foundation, accounted for 80% of the world's output of yellow fever vaccine, with the remaining 20% coming from eleven manufacturers, including a laboratory in Bogota. Technologically obsolete, Manguinhos' other vaccines were not consonant with the agenda of international health agencies.

The first challenge noted with the yellow fever vaccine was its lack of thermal stability, making distribution dependent on a chain of often impracticable low-temperature conditions. The second was subcutaneous injection.

Obsolete equipment hampered larger-scale production (Halstead, 1988, p. 237). The vaccine consisted of a virus attenuated in chicken embryo juice, and the medical literature showed that eggs provoke allergic reactions. Moreover, it had been found that yellow fever vaccine made in Brazil and other countries was contaminated with viruses of the avian leukosis group. The first vaccine free of that contaminant was prepared in 1967 at the Wellcome Research Laboratories. The problem came under control at Bio-Manguinhos following the 1982 introduction of specific pathogen-free eggs. The primary seed lot still contains the avian leukosis virus but its replication time is slower

than that of yellow fever and infected embryos are thus harvested before the virus can develop, leaving secondary and vaccine seed lots free of the contaminant.

The international symposium in Belém approved a program to modernize yellow fever vaccines. The next generation would use in vitro cell cultures, replacing embryonated eggs. On a more immediate basis, both PAHO and the WHO were to support the refinement of lyophilization and methods to render vaccines more stable.

Bio-Manguinhos had just signed a cooperation agreement with the Japanese (1980-84) to use their know-how to produce vaccines against measles and polio. Under Akira Homma, the yellow fever laboratory received more sophisticated equipment, facilities, and protocols, boosting production capacity four-fold. Based on measles vaccine technology, Oscar de Souza Lopes developed a stabilizer for the vaccine and dedicated himself to replacing embryonated eggs with in vitro cell culture. Studies showed that adaptation of an attenuated virus to new cell substrata could occasion changes in its biology and behavior (Halstead, 1988, pp. 236-7). Additionally, the so-called biological markers used to gauge vaccine safety and immunogenicity (i.e., rhesus monkeys and mice) no longer satisfied the experts who were exploring the question from the angle of molecular biology.

Souza cultured the virus from the 17D subsample furnished by the WHO, free of avian leukosis, in primary monolayer chicken embryo fibroblast cell culture. The yield was fifteen times greater than the traditional technique but inoculant consumption was very high. A secondary seed lot, which could feed production in embryonated eggs for ten years, would be exhausted in cell culture in less than one year. The cost would be prohibitive (Maria da Luz F. Leal, interview, Feb. 04, 2001, tape 2, side A).

Bio-Manguinhos' most important partner in this innovative endeavor was Canada's International Development Research Centre. Both institutions took part in the modernization of the production laboratory in Nigeria at the height of an epidemic crisis there.

Mass vaccination began in French-speaking West Africa in the 1940s, using the French neurotropic vaccine strain, generally administered along with the smallpox vaccine via scarification. Some 25 million people were immunized, ending the region's epidemics. In the early 1950s, the same vaccine was used in Central America and

Nigeria, where cases of post-vaccination encephalitis were recorded, prompting the Pan American Sanitary Office to recommend that only the 17D vaccine be administered in the Americas.

People's resistance to taking the vaccine is an important differential in the history of yellow fever in Africa and the Americas. Africa's English-speaking countries were always reluctant to use the French neurotropic virus, and since the 17D vaccine never became applicable through scarification, no efficient immunization policy was ever implemented there. Vaccine coverage was high in French-speaking Africa until decolonization in the 1960s. In September 1986, an epidemic broke out in eastern Nigeria. For the first time, *Aedes africanus* played the role of epidemic vector (Monath, 1991, p. 35). The transmission of yellow fever by *Aedes aegypti* in the western part of that country proved that fears about the potential urbanization of sylvatic yellow fever were not groundless.

Monath and collaborators ascertained that the West African *Aedes aegypti* was a less efficient vector than sub-populations in the Americas (Monath, Miller, and Tabachnick, 1985). The usual explanation for the absence of yellow fever in Asia was weakened, since studies done around that same time showed that its *Aedes aegypti* populations were better vectors than those of Africa (Tabachnick et al., 1985, pp. 1219-24).

In Brazil, a new wave of sylvatic yellow fever rekindled worries about re-urbanization. The number of reported cases rose from three in 1997 to eighty-five in 2000, though we know this is but the tip of an iceberg, with actual mortality rates at about 50% of reported cases.

In the chronic tension between the strategies of combating the urban vector or vaccinating, the second method won out this time. In 1994, the yellow fever vaccine was adopted by Brazil's National Immunization Program (Programa Nacional de Imunizações). Four years later, routine vaccination of children became part of the Expanded Immunization Program. Vaccination grew over 600%, soaring from around 2.5 million doses (2,587,788) in 1996 to slightly over 16 million (16,125,871) in 1999.

On October 16 of that year, a five-year-old girl from Goiânia died days after receiving a dose, administered along with the MMR vaccine. Four months later, on

February 27, 2000, a 22-year-old woman died in the São Paulo city of Americana, eleven days after being vaccinated with the yellow fever antigen alone.

An international expert committee analyzed the two accidents. Passive surveillance of adverse events, introduced in 1998, had recorded 244 incidents, most not serious, out of nearly 35 million (34,693,189) doses administered through March 2000 (FUNASA, May 2000). Two more deaths were identified during retrospective searches.

No antibodies for yellow fever, leptospirosis, dengue 1 and 2, or Hantavirus were found in the girl from Goiânia. The yellow fever virus was, however, isolated from heart, spleen, and skin samples (FUNASA, May 2000). Molecular analysis showed it was not the sylvatic strain, although histopathological tests did reveal changes similar to those produced by the sylvatic virus. Later gene sequencing confirmed that both the little girl and the young woman who had passed away in Americana had been infected by the 17DD strain of virus (FUNASA, May 2000).

Ricardo Galler, of the Oswaldo Cruz Foundation, confirmed that no modification had occurred in the vaccine virus. If a selection mechanism had favored replication of a mutant virus to the point where it superseded the virus that was hegemonic in the genetically heterogeneous population of the vaccine suspension, there would be no way to account for its lethal effect in only two individuals. It was fruit of processes triggered after inoculation and linked to the virus's interaction with still unknown organic peculiarities of these individuals.

The expert committee ruled that universal vaccination was no longer advisable but that the risk-benefit equation justified continued vaccination in risk areas (FUNASA, May 2000, p. 15), as long as a new protocol was designed for the surveillance of adverse events.

On February 23, 2001, on the eve of Carnival, sylvatic yellow fever was confirmed near Belo Horizonte, capital of Minas Gerais. The outbreak was to leave a trail of 15 dead there. Some three million doses were administered in the Belo Horizonte region. On March 18, another death was linked to the vaccine.

Other subsamples of the 17D strain have caused similar problems. News of such adverse advents continues to live alongside alarming news of sylvatic yellow fever knocking on doors in Latin American cities. The deaths that occurred in early 2008 in

San Lorenzo, Paraguay, show that urbanization of the disease continues to hang over our heads, like the Sword of Damocles. We still do not know what biological processes make certain people interact with the vaccine differently from the majority.

Post-vaccination accidents have lent urgency to questions raised since the 1980s about the mechanisms of viral virulence and the immune response of the invaded organism. It is known that the viscerotropism of the wild virus and the neurotropism of the attenuated virus bear a connection to the intrinsic or genetic properties of both the virus and the vertebrate host.

Apparently there are imprecise differences between the South American and African types. Furthermore, today we have evidence that the human immunological response to the disease can be modified by prior exposure to other flaviviruses. This cross-protection is actually one of the explanations behind the surprising fact that yellow fever has not yet urbanized in the Americas.

In May 2001, only three manufacturers had been prequalified by the WHO: Sanofi Pasteur, in France; the Institut Pasteur, in Dakar; and the Medeva Group Development, in the U.K. (www.who.int). Bio-Manguinhos – still the world's largest producer – initiated its certification process in August 1999 and is now prequalified as well. The project to make the vaccine using in vitro tissue culture was resumed there. In experimental batches, yield proved close to that of the vaccine in eggs but a new problem arose. International auditors began rejecting the use of albumin for fear of contamination by the viruses of AIDS or hepatitis B or by the prion that is the agent of bovine spongiform encephalopathy, or mad cow disease. Still found in many vaccines, albumin is apparently essential to the stability of the yellow fever virus in the liquid medium during cell culture. Without this stabilizer, yield would never be commensurate with large-scale production. The project thus faces another impasse.

A new technology may revolutionize the vaccine against the yellow fever agent and other members of the *Flaviviridae* family, including dengue and Japanese encephalitis.

Since the 1983 discovery (by Kary B. Mullis) of the polymerase chain reaction (PCR), which makes it possible to amplify any fragment of DNA and therefore characterize it genetically (Morange, 1998, pp. 2231-2), substantial research efforts

have been aimed at elucidating the molecular bases of attenuation of the yellow fever vaccine virus.

According to Monath (1996, pp. 176-9), one of the biggest challenges in this research are the genetic variations occurring over the long series of empirical passages before arriving at today's vaccine strains. When the polymerase is in action, errors occur that cause small mutations. This is the basic mechanism of evolution, much slower in organisms with genomic DNA, like humans, who have a means to repair these genetic flaws. In a retrovirus like yellow fever, such mutations are accelerated, and in repeated passages through eggs, in vitro cells, or even the cells of the infected organism, a selection may take place within the pool of variants. What prevails is a sub-population, altering known virus phenotypes.

The complete genome sequencing of the 17D virus was decoded in 1985 (Rice, Lenches, and Eddy, pp. 726-33). Other sub-strains were later sequenced, including the one used to manufacture the Bio-Manguinhos vaccine (17DD EP-low) (Galler, Post, Santos, and Ferreira, 1998, pp. 1024-8; Monath, 1996, p. 177).

Infectious clone technology makes it possible to manipulate the genome of the vaccine virus and to engineer mutants, which, in the jargon of biologists, have the curious name of "chimeras." These new living viruses might act as vectors capable of expressing heterologous antigens in the human, thereby triggering an immune reaction against the viruses of yellow fever and other diseases (Monath, 1996, p. 178-9). Galler's group had hoped to include dengue and malaria genes in the yellow fever virus.

This line of innovation continues to be pursued for dengue but has been interrupted for yellow fever. Adverse events associated with the live virus still cultured in embryonated eggs and the potential risks of using cell cultures or chimeras explain the change in course towards vaccines made of dead or inactivated virus by means of different techniques. The vaccine developed by Monath's team, based on chemical methods, is now undergoing clinical trial (Monath et al., 2010, pp. 3827-40), while the vaccine developed at the Oswaldo Cruz Foundation, using the Vero cell method and inactivation through high hydrostatic pressure, is still in the pre-clinical phase.

In January 2011, an agreement was signed by Bio-Manguinhos, the Fraunhofer Center for Molecular Biotechnology, and the U.S. company iBio Inc., targeting another

innovative process. The gene that encodes the main protein of the yellow fever virus, responsible for inducing an organism's immune response, is introduced into leaf cells from *Nicotiana benthamina*, a tobacco species. As the plant develops, its leaves produce a large amount of the antigen to be used in the vaccine. This biofactory method is already being used to produce certain substances, like insulin. If successful, the vaccine will no longer need special embryonated eggs, for which there is still only one supplier in Brazil.

There has also been breaking news from the other yellow fever front, the battle against the potential urban vector, now the transmitter of dengue and responsible for over one million cases in Brazil in 2010 (1,011,548).

At the beginning of this talk, I mentioned the Transgenic Aedes Project, under testing in cities in Bahia since 2011. The program has so far released over ten million male mosquitoes that carry a gene fatal to any larva born from breeding with wild females, as it prevents offspring from reaching adulthood (*O Globo*, Jul. 13, 2012, p. 30). Developed by Britain's Oxitec, the mosquitoes are raised in Brazil's Moscamed biofactory, originally established to produce insects used in the control of fruit flies. In mid-2012, Moscamed opened a new unit, boosting its weekly capacity from about 500,000 transgenic *Aedes aegypti* males to 4 to 5 million. The Brazilian company initially considered using radiation to sterilize specimens, similar to the procedure for flies, but it opted instead for genetic sterilization, engineering transgenic individuals with a technique developed by Oxitec. In 2007, when the British firm approached Fiocruz about a partnership, Foundation personnel raised questions regarding biosecurity, confidentiality, and technology transfer. Field tests were to be contingent on lab tests to verify strain viability. Negotiations were broken off in late 2008 when the British company began mass production in partnership with Moscamed, before any laboratory tests.

Since the early 1950s, numerous proposals have been made to use genetics to frustrate the vector potential of insects implicated in major medical and agricultural problems. According to Benedict and Robinson (2003, pp. 349-55), attempts to control *Aedes*, *Anopheles*, and *Culex* by releasing into the field males sterilized by radiation, chemical agents, cytoplasmic incompatibility, translocation, and other technologies have only met

with some success in small, isolated locations or when something on the order of hundreds of millions of sterile specimens have been involved. On the other hand, Lounibos (2003, pp. 33-43) analyzed tests conducted with *Aedes aegypti* in Kenyan villages in the mid-1970s and concluded that changes in behavior that reduce human-vector contact may offer a simpler alternative than interventions based on genetic control.

Brazilian papers recently announced a new strategy for controlling *Aedes aegypti* (*O Globo*, May 29, 2012, p. 30), developed at Monash University in Australia. Through microinjection, *Walbachia* – an intracellular bacterium found in 70% of the world's insects – is transferred to mosquito eggs. The bacterium is unable to contaminate vertebrates but blocks the virus's action in mosquitoes. When they breed in the wild, passing the bacteria from mother to offspring until infected mosquitoes are dominant, dengue transmission is interrupted.

Today Brazil sets the standard for many countries in *Aedes aegypti* control, largely because of the great role the vector has played in the country's medical and public health history. For over ten years, a national mosquito monitoring network has been working to define protocols and strategies for assessing resistance to pesticides. Brazilian populations of *Aedes aegypti* are resistant to several classes of pesticides, and for this reason should not be used in the mass creation of transgenic mosquitoes.

Control programs once highly centered on the use of pesticides now represent only one approach within broader actions or programs that entail – or should entail – sanitation, education, and citizenship. The investment of limited resources in initiatives that steer the focus away from more sustainable ways of reducing transmission has come under fire by many in Brazilian public health, who also warn about the risk of species that transmit sylvatic yellow fever coming to occupy urban niches.

Here I will close this long overview of the history of yellow fever, a saga with many loose ends still hanging. Beyond our contemporary dilemmas, I see many historiographic challenges. Why, for example, was there such a lag between the great outbreak in Pernambuco in the late seventeenth century and the re-appearance of the disease in mid-nineteenth century Brazil? Why did it stop attacking urban populations in southern Europe? The theory of yellow fever's African origin was conceived from

the perspective that there was only one vector and one vertebrate host. For a time, the discovery of sylvatic yellow fever had put this question back on the table: had coastal cities been invaded from the hinterlands? In this case, the infection might have been raging among indigenes at the time of discovery and then, with the advance of colonization, sylvatic yellow fever could have urbanized.

Narratives about the restructured campaign in the Americas that began in the 1930s confirm that the reassembled gears were soon meshing smoothly, technical and social turmoil aside. Yet accounts anchored in the African experience paint a much less stable or consensual picture of the process. Despite the monumental work *Yellow Fever*, written by Jarí Vainio and Felicity Cutts at the request of the WHO (1998), a global social history of yellow fever – better yet, yellow fever and dengue – has yet to be undertaken, one that encompasses events in the Americas, Europe, Africa, and Asia. And, in my humble opinion, such a history should salvage any positive, still useful experiences from the eradication projects that fell out of favor after the major shift in international health forged at Alma-Ata in September 1978.

Thank you very much for your attention.

Translation by Diane Grosklaus Whitty