An overview of Arbovirology in Brazil and neighbouring countries.

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A HISTORY OF ARBOVIROLOGY IN BRAZIL: BELÉM 1954- 1965

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INTRODUCTION

In 1953, an historic agreement was signed between the Serviço Especial de Saúde Pública (SESP) and the Rockefeller Foundation (RF) to establish a program to study arthropod-borne viruses in the Amazon region. This idea was conceived by a group of scientist administrators at the RF. Robert Morrison, Hugh Smith, John Weir, Max Theiler, and Wilbur Downs had convinced the trustees of the RF that yellow fever was probably not the only serious arthropod-borne virus problem in the world, and that the RF should invest in research to discover others. The RF decided that parternships were needed with governments in the Americas, Asia, and Africa to investigate arboviruses. We sometimes forget in this era of "emerging infections"(Lederberg et al., 1992) that the planners were just as bright and perspective in the 1950's as in the 1990's. Not in their wildest dreams, though, did th RF seges envisage the numbers and importance of the viruses that were to be discovered in the Amazon region.

In 1954, Ottis Causey and his wife, Calista, arrived in Belém with a relatively small budget from the RF and started immediately to establish the site if research which was called the "Belém Virus Laboratory". The history is described in detail by the Causeys (Causey & Causey, 1982). They initially occupied four rooms on the third floor of the Instituto Evandro Chagas (IEC). The Belém city power supply was of insufficient voltage to provide refrigeration, and an 18 KW generator was run six hours per day. In 1958, the city installed reliable power and the RF provided a substation for the RF. That same year SESP offered a partly complete building on the grounds of the IEC. The building was an unoccupied BCG vaccine plant with open cross-ventilation, by today's standards not optimal for biological containment. For 1958 it was quite adequate. The Causey's had the collaboration of IEC scientists Octavio Maroja and D.G. Macedo, and they recruited an excellent cadre of new technical personnel including the top graduates of the University of Pará's Faculty of Pharmacy. One of these, Amélia Paes de Andrade was to become an internationally known scientist in the identification of Brazilian arboviruses, and another, Amazonia Toda was to become a skilled medical entomologist and specialist in tropical culicine mosquitoes. A third addition, recruited later, was an experienced clinician-researcher, Gilberta Bensabath, who had worked as a SESP physician in the interior of the Amazon basin. The new program had the unconditional support of Orlando Rodrigues da Costa, the Director of the IEC.

My own involvement was a fortuitus event. I was a resident in internal medicine at Yale University and was looking to expand my horizons to research. I was recommended by my chief for a Ph.D. program of the newly established Rockeffeler University in New York where the RF had its virus laboratories, and I was invited to lunch with several of the university professors. Max Theiler, Director of the RF Virus Laboratories, was also invited. When Igor Tamm, head of the Ph.D. recruitment asked at the end of the day with whom I wanted to do my Ph.D., I responded "Max Theiler". I had, of course, misunderstood Max's status, thinking he was a university professor. Tamm phoned Theiler who straight away offered me a job to work in Belém.

I was not sure whether to accept the offer. I consulted my father, who was coincidentlaly scheduled to visit Brazil on a foot-and-mouth disease mission. He stopped in Belém on a back to the U.S. to meet the Causeys and Orlando Costa. His advice to me on his return, "Bob, this is the chance of lifetime," and as always he was correct. I put aside my ambitions to earn a Ph.D. and joined the staff of the RF in July, 1958, set about to learn serological techniques and prepare diagnostic reagents, the joined the new section of the arbovirus serology in Belém, in 1959 to work with Amélia Andrade and a visiting scientist from Nigeria, Akinyele Fabiyi. The transfer of technology was instantaneous as the three of us adapted the hemagglutination-inhibition and the complement-fixation tests to the local conditions with the help of a sheep and a goose. These two tests over the next decades were used to survey uncounted numbers of sera for antibodies and to identify literally thousands of arboviruses isolated in the Amazon region.

It is not my intention to detail here the many new viruses resulting from the Belém Virus Laboratory's research and their characterization. This is done well for the first decade of the laboratory operation by John P. Woodall (Woodall, 1967). Over 2,000 strains of arboviruses and arenaviruses were isolated. These constituted 48 serotypes in 18 serogroups, and 8 ungrouped viruses. They included important human disease agents such as yellow fever; Mayaro; eastern, western, and Venezuelan encephalitis; Ilheus; Bussuquara; Saint Louis encephalitis; seven group C and three group Guamá viruses; Guaroa; Oropouche; Piry; and Candiru. This was truly a prodigious accomplishment that became known around the world.

The Belém Virus Laboratory and its staff established several important operational concepts, many of which are described in detail by Calista and Ottis Causey and their colleagues. (Causey & Causey, 1982; Causey et al., 1961) These concepts have since successfully been emulated by many other laboratories.

Mouse colony.

The basic resource for isolation and identifying new arboviruses was the baby mouse. The colony was under the able supervision of Calista Causey. Calista was a volunteer worker, formely a professor of Bacteriology at Johns Hopkins University, who trained a highly efficient staff. The colony was easily capable of producing 100 litters per day. Mice were outbred and not specific-pathogen free. Adventitious viruses were recognized and did not interfere with the program. The mice were healthy and were regularly transported by van to the field, sometimes long distances. In order to maximize production, female mice were segregated at birth and those needed for breeding were returned to the production colony, while the male mice were used for inoculation. Without the mouse resource the project could not have functioned.

Carpenter shop.

Another basic resource was a resident carpenter who constructed hardwood rodent box traps, various mosquito traps and other field apparatus. Most of this work was done manually, but by the end of the decade he began using power tools. Many clever original inventions had their origin in this shop.

Sentinel animals.

In november 1954 *Cebus apella* monkeys were obtained from an island off the coast. Because of their insular origen the monkeys were free of antibody to the arboviruses endemic in the Belém area. These animals were exposed in the Oriboca Forest where they early on became infected with Bussuquara, Venezuelan encephalitis, group C, and group Guamá viruses. Ottis Causey devised a clever method to feed the monkeys so that they did not consume or discard all of the food at once, and could be left happily alone in the forest for several days suspended off the ground in wide-mesh cages. Pellets of food were placed in coffee cans that had an opening just large enough for the monkeys to insert a finger and remove a single pellet at a time. After eating enough food to satisfy their hunger, the monkeys tired, and started again only the next day to remove more pellets. Monkeys were bled twice a week to test for viremia which was detected in 7.4% of 29,977 specimens.

From 1955 on, adult and then baby mice, were used as sentinel animals. The mother and six babies were placed in small large-mesh cages under an aluminum hood to keep out rain and predators. This hood was so successful that it was adopted by other arbovirus programs and came to be known as the "Causey hood." In the first two years of use, 32.1% of sentinel infant mice yieldeld and closed units exposed in series in order to find out what time mosquito transmission occurred.

Transmission of arboviruses by wild-caught mosquitoes

Mosquitoes were routinely captured, identified, triturated, and inoculated intracerebrally to baby mice in order to isolate arboviruses. This technique was effective but did not tell whether or not the mosquito could transmit. To answer this question forest-caught mosquitoes were identified, kept alive, and each species placed in a separate cage to detect transmission to sentinel mice exposed within the cage. Several arboviruses were isolated and their vector determined by this method (Toda & Shope, 1965).

Capture-mark-bleed-release-recapture of rodents and marsulpials.

Hugo Laemmert of the Insituto Oswaldo Cruz (IOC) supervised an IOC field team in Belém. After Laemmert's death, this team remained with Ottis Causey who instituted a capture-mark-bleed-release-recapture program to study rodent-arbovirus interaction in the IPEAN and Utinga Forests. Initiated in 1962, the recapture program was contined when I became laboratory director in 1963, and when John P. Woodall succeded me as director in 1965. The team was highly skilled and could withdraw blood by needle from the heart with rarely a death of the animal.

From July 1962 to November 1964, 1389 animals were sampled 8750 times (Shope et al., 1988). Most of the animals were rodents that constituted the vertebrat reservoir hosts of Venezuelan encephalitis, Bussuquara, and several group C, group Guamá, and phleboviruses. The concept was simple and incorporated many of Hugo Laemmert's and Ottis Causey's ideas. A grid design permitted calculation of the animals' home ranges and their preferred habitat. Silk strings were attached to rodents as they were released, so that their nesting burrows could be located as they returned to them. In order to find what time the rodents were trapped, alarm clocks were rigged by Ottis Causey to stop the clock when the trap was entered. A string going from the trap door to the flywheel stopped the clock. The approximate age of animal was calculated from age-weight curves. Rectal temperatures were recorded and related to viremia presence or absence. Sera were tested for antibody and viremia, and dates of infection were recorded (Shope et al., 1966)

Concept of ecological separation of virus transmission cycles in the forest

Traps were also hoisted on wooden plataforms into the forest canopy. A different set of animals, many marsupials, was discovered in the tree tops, and different viruses such as Oriboca and Apeu in group C were isolated. These observations reinforced the concept that separate cycles of transmission could be going on at different levels in the same forest sites.

Thus a complete virologic history was analyzed together with the complex ecologic profiles of each of the 1389 animals. These data, together with data on the ecology of mosquitoes permitted a refined analysis of the epidemniology of each of the arboviruses in the area.

Capture and recapture of birds.

In 1964 Philip Humphrey, curator of birds of the Smithsonian Institution, Washington, DC, instituted a recapture program of birds at the forest sites where mammals were being sampled. Not surprisingly, a different set of viruses including eastern encephalitis, western encephalitis, Saint Louis encephalitis, and Turlock viruses were repeatedly recovered. (Shope et al., 1966) This program was continued after John P. Woodall succeded me in 1965 as laboratory director.

The field laboratory in Amapá Territory

In 1964, Francisco Pinheiro established a field laboratory in Amapá Territory in collaboration with the aluminum mining company, ICOMI. This was the predecessor of many outreach programs where the laboratory carried out research on site away from Belém. The *Arenavirus*, Amapari, was isolated and its transmission in rodent studied. This virus is now known to be genetically very closely related to Junin, Machupo, and Guanarito, all three of which have high case-fatality rates in humans. Amapari virus is not known to cause any illness in people, an unexplained mystery given the close genetic relationship to the other serious human pathogenic arenaviruses.

People as indicators of arbovirus transmission.

Persons who come to an endemic area from other geographic regions are often non-immune. The staff of the Belém Virus Laboratory recognized this principle early in the program and designed surveillance accordingly. Migrant laborers from Ceará or from the Atlantic coast were employed in the Oriboca Forest and other forests to clear land for agriculture. These were effective human sentinels and yielded much of the early information on human clinical illness with arboviruses. Ottis Causey thought that Japanese immigrants brought to Brazil to colonize land on the Guama River would also be good sentinels. He discovered, however, that the Japanese did not frequent the forest and that the better sentinels were the Brazilian workers who cleared the land to the colonists later cultivated.

He seized the opportunity in 1960 to study febrile workers on the Belém-Brasilia highway construction. This again proved a fruitful source of arboviruses.

Ourbreak investigation.

Outbreaks of febrile disease provided an opportunity to find arboviruses in humans and domestic animals. These early studies revealed activity of yellow fever, Mayaro, eastern encephalitis in horses, and Oropouche viruses.

The investigation of the Oropouche outbreak in Belém in 1961 represented the maturation of the laboratory (Pinheiro et al., 1962). This was a large epidemic involving thousands of persons residing in the Belém districts of Marco and Pedreira. It was an epidemic that would have escaped notice if the laboratory had not been there and been prepared to isolate virus and carry out serologic tests. It occurred while Ottis and Calista were in the U.S. on home leave and thus demonstrated that the resident staff were fully competent to investigate such an outbreak.

Training and infrastructure

In the absence of the Causeys, the lead in the Oropouche investigation was taken by Maria and Francisco Pinheiro. Maria was an immunologist trained in São Paulo; Francisco had joined the laboratory as a volunteer when he was still a medical student at the University of Pará. His presence was a direct result of a plan by Ottis Causey and the IEC Directors, initially Orlando Costa and later Manoel Bruno Lobo, to attract and hold the best young Brazilian scientists at the IEC. Francisco subsequently carried out post-doctoral training at Yale University sponsored by RF. He established the tissue culture laboratory at the IEC, became Institute Director, and subsequently has had an illustrious career in Washington, DC at the Pan American Health Organization.

The Belém Virus Laboratory has also been a training site for non-Brazilians. It had a tremendous posaitive influence on my own career, helped launch John P. Woodall to many years of service with the World Health Organization, and since the 1960's has continued to foster scientists from many different nations.

The success of the Belém Virus Laboratory reflects the early philosophy of the RF and specifically of the Program Director, Wilbur Downs, in all of the arbovirus laboratories around the world. Downs intended that the programs be joint efforts to culminate in a self-sustained national host laboratory. The RF staff members in Belém were regarded by the RF as important but transient partner visitors, and they were evaluated and rewarded primarily on the basis of the success of their Brazilian counterparts.

Networking with other laboratories and agencies

The most effective research is conducted with collaborators. Essential to the success of the Belém Virus Laboratory during its first decade was the network of supporting and collaborating organizations. The financial support and nurturing of the RF is clear from the preceding discussion. The RF invested $30 million to the overall arbovirus research between 1952 and 1970, when it gradually withdrew from the program (Theiler & Downs, 1973). The budget was divided among the central laboratory in the U.S. and the field sites in Brazil, Trinidad, Colombia, Egypt, California (U.S.), Nigeria, South Africa, and India. The Belém Virus Laboratory collaborated actively in the first decade with the Gorgas Laboratory and the Middle America Research Unit in Panama, the University of São Paulo, the University of Brazil, the University of Pará, and the Instituto Oswaldo Cruz. Other Brazilian agencies contributed money or personnel including the National Department of Rural Epidemics, the SPVEA, the SPI, the National Research Council, and the National Museum.

This complete only the first chapter in the illustrious history of the arbovirus program in Belém. The virus laboratory and the IEC subsequently expanded their collaboration to include other organizations such as the U.S. military and the French Government, and have studied arbovirus problems associated with extensive road building, deforestation, and dam construction in many sectors of the Brazilian Amazon.

Formidable future challenges face this dynamic team, not the least of which is the renewed threat of *Aedes aegypti*, urban dengue and yellow fever.

REFERENCES

CAUSEY CE, CAUSEY OR - 1982 - Development of arbovirus studies in the Amazon Region. In: International Symposium on tropical Arboviruses and Hemorrhagic Fevers. p.13-20. *Academia Brasileira de Ciências*. Rio de Janeiro.

CAUSEY OR, CAUSEY CE, MAROJA OM, MACEDO DG - 1961 - The isolation of artrhropod-borne viruses including members of two hitherto undescribed serological groups, in the Amazon region of Brazil. *Am. J. Trop. Med. Hyg. 10*:227-249.

LEDERBERG J, SHOPE RE, OAKS SC Jr. - 1992 - Emerging Infections. *National Academy Press*. Washington, USA.

PINHEIRO FP, PINHEIRO M, BENSABATH G, CAUSEY, SHOPE RE - 1962 - Epidemia de vírus Oropouche em Belém. *Rev. Serv. Esp. Saúde Pública, 12*:15-23.

SHOPE RE, ANDRADE AHP, BENSABATH G, CAUSEY OR, HUMPHREY PS - 1966 - The epidemiology of EEE, WEE, SLE and Turlock viruses, with special reference to birds, in a tropical rain forest near Belém, Brazil. *Am. J. Epidemiol. 84*:467-477.

SHOPE RE, WOODALL JP, TRAVASSOS DA ROSA A - 1988 - The epidemiology of diseases caused by viruses in groups C and Guamá (*Bunyaviridae*). In: MONATH TP (ed.), *The arboviruses: Epidemiology and Ecology*, v. III, p.37-52, CRC Press, Boca Raton, USA.

THEILER M, DOWNS WG - 1973 - The Arthropod-borne Viruses of vertebrates. *Yale University Press*. New Haven, USA.

TODA A, SHOPE RE - 1965 - Transmission of Guamá and Oriboca viruses by naturally infected mosquitoes. *Nature 208*:304.

WOODALL JP - 1967 - Virus research in Amazonia. *Atas do Simposio sobre a Biota amazônica 6(patologia)*:31-63.