Mosquitoes and yellow fever virus

by

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ARE URBAN AND JUNGLE YELLOW FEVER TWO DIFFERENT DISEASES?

The first references to the presence in our Continent of a new form of yellow fever, different from that known here for two centuries, because it prevailed in the jungle instead of occurring in the cities, it was able to infect people during day-time and it was transmitted by jungle mosquitoes since Aedes aegypti did not exist in the foci, date from some 60 years ago. This type of jungle yellow fever was very different, on account of these characteristics, from the classic disease, known up till then, which prevailed in the cities of the coast and of the interior, where Aedes aegypti existed as a vulgar and domestic mosquito.

The jungle form of yellow fever was for the first time appointed, in America, in 1887, by Camio y Ortiz in small foci of the jungle near the localities Abapó, El Espino and Muchuri, south of Santa Cruz de la Sierra, in Bolivia. More or less ten years later on, we meet with references of Lutz to cases in the jungle of Funil, near Campinas, and also in the zone of Taboão, near Taubaté, the Sanitary Service of the State of São Paulo found out, in 1904, cases of yellow fever, without discovering Stegomyia in the places where the patients were living. In 1907 a very characteristic focus of jungle yellow fever, which became classic, was reported to exist in the mines of emeralds of Musso, Colombia, where up till now the disease maintains itself, although with interruptions. This focus has been very well studied by the colombian physicians Franco, Martinez, Santa Maria and Toro de Vila, who not only identified the prevailing disease as yellow fever, but affirmed also that it was acquired during day-time, having as transmitting agents jungle mosquitoes and Stegomyia. Later on it was got known that there had been, on the part of Franco, some confusion as to Stegomyia, since that mosquito does not exist in Musso, as has been proved by the researches, made by the Rockefeller Commission, that confusion

565
having probably been caused by some striped mosquito of the jungle.
The focus, observed in the mines of Musso, is especially interesting for
being completely isolated, without any contact of people, working there,
with any other near point where eventually urban yellow fever might
prevail. These first papers, referring to the existence of yellow fever
of sylvester character in these limited foci, did not call the attention
of the physicians or hygienists of that time and the matter fell into
oblivion up till recently.

Here, in Brazil, nothing more was observed during more or less
30 years, after the references to São Paulo. On the other side, thanks
to the anti-stegomyical measures, taken in the cities by the Sanitary
Service, the classic urban yellow fever disappeared nearly completely
from the American Continent, continuing only very feebly in the zone
of North-Eastern Brazil, where it subsisted in its rural form, but always
dependent on the presence of Stegomyia. The decrease of yellow fever
during a large period made the matter loose a great part of its original
interest, and even at a certain moment it was accepted that it had
wholly disappeared from the Continent. Unhappily that supposition did
not last for much time, and shortly the evil re-appeared in its common
urban and rural forms, due to the sending of troops, not immune against
yellow fever, to regions of the north-East of the country, where, accom-
panying the military forces in movement, it attained the valley of São
Francisco, attacking the city of Pirapora, State of Minas Gerais, in 1926,
where it caused an urban outbreak, subsequently extinguished by pro-
phyllactic measures against Stegomyia.

In 1928 there appeared suddenly once more the classic yellow
fever in the city of Rio de Janeiro, where it had disappeared for 20 years,
spreading subsequently, always in urban form, over 44 localities of the
State of Rio de Janeiro and over some ones of the State of Minas Gerais,
São Paulo and Pará, but disappearing again completely the next year,
after having caused some thousands of cases in the city of Rio de Janeiro
and in other places of the country.

At that time no reference has been made to the occurrence of
the jungle form, as sequel of that large outbreak of classic urban yellow
fever, what is very surprising in view of the fact that on that occasion
yellow fever spread in not less than 44 localities in the State of Rio de
Janeiro, and that in many of these the jungle extends itself to the urban
limits. It ought to be noted that at this time all the zones of that State
were severely watched by the sanitary authorities, who discovered all
these numeros urban foci and from whose attention no unusual disease,
eventually prevailing in the forests, could, no doubt, escape. At that
time there was also no verification of mortality among howling monkeys in the forests, what nowadays is very common and a rule without exception in the places where jungle yellow fever is prevailing for the first time.

In 1932 references to the existence of jungle yellow fever began again to circulate with the discovery in the Valle de Chanaan, State of Espirito Santo, of cases of a suspected disease, in full jungle, in a region of isolated dwellings, but without the presence of Aedes aegypti. At that time the Rockefeller Commission identified perfectly the disease as yellow fever by its clinical manifestations and by means of isolation of the virus in rhesus monkeys and mice.

Since then the matter became very important, because the disease showed a wide and unexpected spread in several zones, especially in North and Southern Brazil, in Colombia and Bolivia, and later on in Venezuela, Paraguay, etc., where it has been accurately studied on the part of the Rockefeller Commission, by the experts of the Oswaldo Cruz Institute and by several physicians, interested in this matter.

Since 1935 up to the first months of 1939, the outbreaks of jungle or sylvan yellow fever succeeded themselves in the Southern States of Brazil, in Matto Grosso, Goyaz, Sao Paulo, Minas, Paraná, State of Rio de Janeiro, in the South of Espirito Santo, and in the State of Santa Catalina, and without any fear of error, the cases, there appeared, may be valued at ten thousands, if we take into consideration that the mild manifestations of the disease, which, as a rule, escape from exact diagnosis, are 10 to 20 times more frequent than the cases, which clinically can be diagnosed with certainty. There must have been, at least, 2 thousand fatal cases during that period, when we take into consideration that the cases, diagnosed by viscerotomy and by necropsy, do not represent their total, since not all the cases, occurring in the interior, can be fiscalized systematically by physicians or sanitarins.

The nearly unanimous agreement of the physicians, who have carefully observed the jungle disease, and specially of those who, times ago, had to do with the old yellow fever of the cities, is that the jungle disease does not differ from the urban disease, transmitted by Stegomyia neither in its clinical aspect nor in its anatomo-pathological or histo-pathological lesions, nor by its immuno-reactions etc., and that only its epidemiology is very different. Some authors have the opinion that the jungle-disease is milder than the disease, anciently verified in the cities, but that opinion is not confirmed by observations of the actual outbreaks, and, even if it were so, the argument would not be conclusive in order to consider, only for that reason, the two diseases different one from another, since, in the same way, scarlet fever and diphtheria in Rio de
Janeiro are not different from those of São Paulo, although being much milder in the first named city than in the second one. Some other authors have the opinion while looking for differences between the urban disease and jungle yellow fever, that this last one does not show any tendency to attack a locality where Stegomyia are found. To-day it has been perfectly verified that the jungle virus develops itself in the mosquitoes of the jungle and also in Stegomyia, and therefore there would not exist any obstacle to the jungle-disease to invade the cities, when these places receive patients, capable of infecting mosquitoes and their index of Stegomyia is high and their conditions of temperature favour the transmission of the disease by *Aedes aegypti*. For the rest, what has been demonstrated by observations, made in localities where such conditions prevailed, is exactly the passage of the jungle virus to the cities, as happened in Cambará, State of Paraná, in Figueira, State of Espírito Santo, in Theophilo Ottoni, State of Minas Geraes, and in Presidente Wenceslau, State of São Paulo. In this last named city the index of Stegomyia which was high in the occasion of the outbreak of the jungle-disease in its environs, there were formed in the beginning of 1937, two urban foci, which caused several cases in people who had not left the locality, and in one of which the disease irradiated itself, in the old classical way, from a boarding-house, which had received patients in the infecting phase of the disease, originating from the near jungle, as has been very well verified by Dr. Idt Pontes, chief of Yellow Fever Service of São Paulo, in the sector, to which the said locality belongs. That nowadays these penetrations of jungle yellow fever are less frequent in the cities of the zones where it prevails, is due, for a great part, to the rapid decrease of their index of Stegomyia, obtained by the prophylactic services against yellow fever, started immediately on its outbreak, and frequently also to the somewhat low temperature, which does not permit transmission of the virus by Stegomyia, but is still sufficient to allow its transmission by jungle mosquitoes, which are less exigent as to thermic degrees not only for living but also for transmitting the disease.

It is very surprising that, when outbreaks of urban yellow fever occurred every year in several localities on the coast and in the interior, in which the disease was very well known by physicians and even by laymen, it has not been frequently signalized in the jungle, with exception of the rare and very limited foci, in which it had primarily been discovered many years ago, what proves perhaps the still imperfect adaptation of the urban fever to the jungle mosquitoes, the foci remaining, therefore, limited.

The woods and the farms were then frequented by many people
of the cities, who went there for business or for pastime, as for instance, hunting, walking or fishing, etc. It is worth mentioning that in the Southern States of Brazil, specially in the States of Rio, Minas and São Paulo, all that tremendous struggle of cleaning virgin forest for planting billions of coffee-trees during a period, in which the jungle and its animals were much more abundant than nowadays, has been done without there having been signalized outbreaks of jungle yellow fever. The farms of the plateau of Minas, and those of São Paulo were considered healthfull places, there not being any notice of having appeared there large outbreaks of yellow fever with the characteristics of the recent epidemics although the cities and the surrounding localities had suffered repeated and decimating epidemics of urban yellow fever, as happened, for instance, at Cantagallo, Vassouras, Valença, Juiz de Fora, Barra do Pirahy, Barra Mansa, Areias, Campinas, Ribeirão Preto, Rio Novo, São Simão, Rio Claro, Sorocaba and in so many other ones. Besides, since 1888, with the abolition of slavery in Brazil, there was established a large course of immigration of European people, which brought, within little more than a decennary, to the farms of the interior more than one million of individuals, sensitive to yellow fever. In the rural zones of the South, these immigrants, although always in contact with the jungle on account of their occupations, never were victims of jungle yellow fever while, if they went to and stayed even for only one night in the cities, where the disease prevailed, they were frequently attacked by the urban disease, which killed them on large scale. The forests and the farms served in earlier times exactly as refuge to the population of the cities, invaded by yellow fever, when the disease became more intense, and, notwithstanding, there was not any propagation of the disease to the mosquitoes, to jungle animals or to sensitive individuals, living in the rural zones.

When, on occasion of the flights of these masses of people, there arrived individuals, attacked by the disease, at the rural zones, the disease extinguished itself without any sign of propagation to the forests, what fatally would have been observed, if the urban virus were easily adaptable to transmission by jungle mosquitoes. It ought also to be noted that at that time no great mortality of howling monkeys (Alouata) was observed, what to-day is very common and is a certain index of the existence of the jungle disease, to which they are sensitive. Such epizootics, if then occurred, would have fatally been noted by the peasants and hunters, who, as good observers of the animals they kill, would have signalized them, as it is being done nowadays, in every part where the jungle disease appears for the first time. To-day exactly the con-
trary is happening and reported, by the inhabitants of the rural zones, to great mortality of monkeys as being constant in all the foci of jungle yellow fever. The great mortality of howling monkeys proceeds nearly always even the appearance of human cases. In forests, where jungle yellow fever is prevailing, these howling monkeys, if they do not die, remain for a long period, silent, as if terrified by the unusual disease existing within them; and this fact would also fatally have called the attention of the nearby living inhabitants, accustomed to their nearly daily howling.

Not less evident was the absolute absence of immunity, formerly observed, to urban yellow fever, and nowadays, to the jungle disease, on the part of the inhabitants of the forests and of the farms of the interior in Southern Brazil, in São Paulo, in Minas, in Rio de Janeiro, in Paraná, and in Santa Catharina, whatever the age, proving therefore, that so much in one period as in the other one, neither the urban yellow fever propagated itself to the forest, nor the jungle yellow fever of to-day had anteriorly disseminated itself there.

The sons of the farmers, the students and all the rural people who came in past times from the interior of Minas, São Paulo, Paraná, Santa Catharina etc., to Rio or Santos, where the disease was endemic or whatever other focus where the disease existed, paid heavy tributes to yellow fever; they had, however, passed great part of their life in frequent contact with the forests of the regions where they were born, without having thus acquired any immunity against yellow fever, what surely would not have happened, if they had previously suffered an attack of the disease, be it the mildest possible.

Remarkable is the contrast between the sensibility to yellow fever, observed among the inhabitants of the rural zones of Southern Brazil, where the disease prevailed only in the cities, and the high degree of resistance of the rural populations of the North-East of Brazil, where, beside urban yellow fever, it existed also the rural form, transmitted by Stegomyia, and very well observed and described by Dr. Sebastião Barroso some 20 years ago. That contrast finds its explanation in the fact that the urban yellow fever of the South did not show any tendency to attack the rural zones, where no Stegomyia were found, while in the North-East the same high temperature of every day and the custom of the population of accumulating water within the house, facilitate the abundant proliferation of Stegomyia in the rural dwellings, allowing the yellow fever, transmitted by that insect, to last and to spread, conferring to the inhabitants the observed high degree of resistance. A consequence of the thus acquired immunity is the fact that the inhabitants of the
rural zones of North-Eastern Brazil could in early times visit with impunity, cities where yellow fever prevailed, while the inhabitants of analogous zones of the South died in large number, victimized by the disease, when they ventured to frequent the localities, where it prevailed. As we had the opportunity to observe in the surroundings of São Paulo and in the chain of mountains of Mogi das Cruzes where no malaria exists, the inhabitants of this zones, recently invaded by sylvan yellow fever, do no more refer that in other epochs there had prevailed any nosological entity with the the aspect of that actually observed, being also anteriorly unknown the nowadays observed great mortality among howling monkeys.

If the jungle disease were frequent in the forests of the Southern States nowadays contaminated, it would anteriorly have caused, as it is doing to-day, great rural epidemics and would continue so after the extermination of the urban fever, showing now and then not only jungle outbreaks, but would also invade the nearby situated cities with the existing high indices of Stegomyia, at the time in which the transmission by mosquitoes was still ignored. At this time, such fact, which would represent a common epidemiological aspect, has not been observed and only now this was noted as a consequence of the adaptation of the yellow fever virus to the jungle mosquitoes, and therefore it became frequent in Southern Brazil, under the aspect of the jungle disease.

Worth of special reference is the fact that urban yellow fever did not give way, for instance, during the epidemics of 1928 and 1929, to an outbreak of jungle fever in the State of Rio de Janeiro, where its presence had been observed in 44 localities of the interior, causing thousands of mild cases, beside some hundreds of serious ones, to judge from the results of the protection test, realized by the Rockefeller Commission at Cambucy, Magé, Santo Aleixo, etc., where the not very apparent cases comprised more or less 60% of the inhabitants of these localities, showing the immunity acquired by a previous attack of yellow fever. By the way, it ought to be noted that at Magé and Santo Aleixo, and in some other localities of the State of Rio de Janeiro, the jungle extends itself practically to the urban perimeter, and that these localities are situated in hot zones with many mosquitoes. In spite of all these favourable conditions, no invasion of the forests by urban yellow fever took place at that time in these localities nor in other parts of the States of Rio, Minas, São Paulo, etc., nor the mortality of howling monkeys (A. ludens) was noted, notwithstanding the presence of extremely favourable conditions for the transformation of the strictly urban disease into a jungle one. On the other side, in the North East of Brazil, where yellow fever always existed in its rural aspect, invading the small settlements of the
interior, and is only transmitted by Stegomyia, it no more did transform itself into jungle disease, in spite of the presence, in many parts of this zone, of forests with mosquitoes and jungle animals, to which the disease could easily be propagated.

From all these facts there results that not only in the urban zones but also in the rural ones and in the forests yellow fever may prevail with different epidemiological aspects but being always the same morbid entity from the point of view of its clinical manifestations, of the lesions and of immuno-reactions, etc. Urban and rural yellow fevers are both only transmitted by Stegomyia, not showing, therefore, any tendency to propagating themselves to the forests, while jungle yellow fever must be considered a consequence of the adaptation of the virus to other mosquitoes than *Aedes aegypti*, which, for living habitually in the forests, made possible its propagation to them in the actual jungle form. May be that this adaptation of the urban virus to the mosquitoes do not date from much time at least, in the American Continent, since the first foci of jungle yellow fever were observed some 60 years ago, and only now the disease seems to take greater increase, for being favoured by the intense human circulation, observed in the interior of the country, after the construction of roads and the introduction of automobiles. It is difficult to say if this adaptation of the virus to jungle mosquitoes was done slowly, or abruptly by mutation. It is possible that this adaptation in higher degree be still going on, because some jungle mosquitoes, as we have, for instance, observed for *Psorophora discrucionis*, conserve the virus only for 8 days; other authors verified that, for instance, in *Mansonoida* the virus multiplications itself very well, but is not transmitted by the bite of the insect, while *Aedes scapularis*, *fluviabilis*, *leucocelanus*, *Hemagogus capricorni*, etc., are well infected and transmit the disease by biting, as happens with Stegomyia.

As to *Aedes scapularis*, it ought to be noted specially that Marchoux, Simond & Salimbeni, at Rio de Janeiro, were not able, in the beginning of this century (Ann. Inst. Pasteur, 1906 — Tome 16) to obtain the transmission of the classic urban virus by this mosquito, while nowadays it is known that this species occupies a place among the jungle mosquitoes which infect themselves and transmit, by biting. It is to be noted that the experiences of Marchoux and his colleagues were made under the best conditions, since the *Aedes scapularis*, with which they had made their experiences, sucked the blood of a patient of yellow fever in the infecting phase, and remained afterwards for 13 days at a temperature of 27°, and subsequently, 4 of these insects bite without any
result a not-immune individual, as was stated, because this patient was ulteriorly submitted to amarillic infection by Stegomyia, and got yellow fever.

Before this adaptation of the jungle virus to various mosquitoes, Stegomyia was probably the only transmitting agent of yellow fever, as seems to be demonstrated by the fact that since 2 centuries up to recent date the propagation of the disease to the forests had not been observed, what would been, however, very easy discovered, as it was the case in the first foci, observed in the isolated and distant inland of Bolivia, and in that very limited focus of Musso, etc. Now then, if in these remote places yellow fever was immediately diagnosticated, although the cases not being many, the more reason there is to believe that it would have been diagnosticated, if it had spread in the coffee-zone of Southern Brazil, which comprises thousands of kilometers with numerous cities, where the urban disease prevailed periodically in great outbreaks during many months without interruption, and where there were many competent physicians, who knew very well the disease. The forests were at that time intensively explored for the planting of coffee-trees, and, if among the numerous groups of wood-cleavers jungle yellow fever had appeared in its commonly observed aspect, the disease would no doubt have been surely diagnosticated, since it would have called the attention of all people, laymen and physicians, specially in the healthy zones of the States Rio de Janeiro, Minas Geraes and Sao Paulo, where there was no serious tropical malaria to be confounded with that disease. May be that the actual presence of yellow fever in the forests shocks a little those who do not pay attention to that adaptation of the virus to jungle mosquitoes, but in view of the old doctrines of miasmatic contagion there would not been any difficulty in admitting that the disease, which prevailed in the cities, could also constitute foci in the forests. Well then, in spite of all these favourable conditions present in very extense zone of Brazil on account of the great development of the coffee-culture, in 3 of the Southern States, there does not exist any reference in these regions, to outbreaks of jungle yellow fever during the long period of more than half a century up till recent times. The area comprised hundreds of thousands of square kilometers, covered by dense virgin woods, with farms, where more than 1 million of people laboured, where mosquitoes and sensitive monkeys (Alouatta) were not failing, and where an intense intercourse existed between the cities of that region and the rural zone, and, although in many of these nuclei of population, yellow fever prevailed periodically again and again, the urban disease did not propagate itself to the woods, nor disseminated there itself transformed into jungle disease. Nowadays
that there are much less woods and monkeys, the population of numerous zones being more scanty on account of the extintion of many coffee plantations, we see that in absence of the urban disease, jungle yellow fever to invade the whole extense zone, which formerly was immune, as is shown by the great sensitiveness of its rural inhabitants to yellow fever, when they went to the cities where the disease prevailed, what would not have happened, if they had previously suffered even the slightest attack of yellow fever in the forests.

The adaptation of urban amarillic virus to jungle mosquitoes, allowing its propagation to the forests, is quite acceptable in an era, in which we assist at adaptations and disadaptations of malaria parasites to certain Anophelinae in several parts of the world; this matter has been very well studied with respect to the varieties of Anopheles maculipennis, the differential characteristics of which are minim, sometimes only perceptible in the eggs of these mosquitoes. Even these smallest structural modifications are sufficient to produce profound alterations in the epidemiology of malaria with the consequence that in certain regions the disease develops itself largely and disappears in other ones, where it had already prevailed intensively, according to the adaptability or not, of the hematogea to the existing variety of Anopheles maculipennis. Several other adaptations and disadaptations of parasites and virus to their transmitting agent have certainly occurred in course of time.

On account of the prophylactic measures against Stegomyia, applied on large scale in Brazil by the Yellow Fever Service, the urban and the rural diseases, transmitted by Stegomyia, have been very much reduced, if not wholly extinguished, their last great outbreaks being those, appeared in the Capital of the Republic, in the State of Rio de Janeiro and in other States of Brazil in 1928 and 1929. The jungle or sylvan fever extends itself, on the contrary, every time more not only in Brazil but also in other South-American countries and in Africa, constituting now a serious menace to the world, since its propagation to other still immune american, european and asiatic regions, in which exist urban and jungle mosquitoes capable of transmitting, is not impossible. Now that the human intercourse becomes every time more rapid and more frequent thanks to the automobile and the airplane, the danger becomes every day more pressing. It is, however, to be expected that the prophylactic services against the disease become, in their turn, more efficacious and that, above all, the vaccination, done at large scale, make finally stop the march of this terrible scourge of mankind.
II

THE INFLUENCE OF TEMPERATURE ON THE EPIDEMIOLOGY OF YELLOW FEVER, TRANSMITTED BY JUNGLE MOSQUITOES AND BY Aedes Aegypti.

Those who are observing the evolutions of the recent epidemics of jungle yellow fever of 1934 to 1937 in the elevated zones of the plateau, in the States of São Paulo, Goyaz, Minas and Paraná, at altitudes of 500 to 1.000 meters, were surprised by the fact that no foci of yellow fever were formed in the cities, infested by Stegomyia, and which received and hospitalized patients, still in the first three days of the disease, from the woods.

Some authors interpreted that occurrence as a proof that the extra-urban, jungle yellow fever was different from the classic urban yellow fever, but nothing authorizes such supposition, as the own biology of Stegomyia furnishes the exploration of this epidemiological aspect, a little perplex, no doubt, but perfectly comprehensible, when we take into consideration that the vital optimum of Stegomyia lies between 27° and 32° and that from 25° downwards it begins to show a more reduced activity and retardation of all the functions, represented by less activity to bite, by the delay of ovulation, of the posture, of the larval phase, of the nymphal activity, etc. For instance, between the temperature of 22° and 23° the posture may last 20 to 30 days, the larvae having a very slow evolution; under 17° we may say that all the vital activities of the mosquitoes stop and it is condemned to disappear.

Stegomyia is especially a mosquito of places of high temperature, oscillating between 27° and 32°, being always very sensitive to low temperature with the consequence that in climates of elevated regions of the States of São Paulo, Goyaz, Minas Gerais and Paraná, where summer temperature oscillates between 22° and 24° and rarely more, Stegomyia lives in conditions which are not very favourable to its life cycle. They show, therefore, less tendency to bite, their posture is retard till 20 and more days, from their eggs larvae are born after 20 days, and their larval and nymphal phase are also delayed, as has been shown by the classic experiences of Marchoux, Salimbeni and Simond. In such conditions, when the mosquito sucks once, taking 18 to 20 days for the first posture, it is already, when the posture terminates, at the end of life, not returning to bite. On the other side, low temperatures exercises an accentuated action on the multiplication of virus in the mosquito, reducing it and impeding its arrival, at the salivary glands and its trans-
mission by the bite. At high temperatures it is possible that Stegomyia transmit the virus within 4 to 5 days, but the interesting observations of Davis show that at 25° the mosquito wants 8 days for transmitting yellow fever, at 23° this term is of 11 days; at 21° it wants 18 days, and at 18° it practically does not infect any more, and after 30 days its biting is inefficacious, although its virus remain in a state of hibernation, since, it conserved for more than 6 days at 36°, it reassumes again the capacity of transmitting yellow fever by sucking.

The life-time of the mosquito in liberty, in the climate of São Paulo, suffering all the dangers and intemperies and the influence of the great variations of temperature, which oscillate between 12° and 20° at the same day, does not exceed, in our opinion, even in the best conditions, 20 to 25 days.

We know that the vitality of the mosquito is very much influenced and prejudiced by these falls of nocturnal temperature, very common in São Paulo and in other places of the Brazilian plateau during summer when the thermometer attains 16 to 18°, these frequent days of low temperature being intercalated by hot days after rain or after chilling winds of the South. The average of temperature, generally low, which prevails on the plateau of São Paulo, of Minas, of Goyaz, and of Paraná, even during summer, and these falls of nocturnal minims to limits, which shorten the vitality of Stegomyia, retard all its functions and its necessity of biting and do not allow the virus to multiplicate itself, or, if so, only slowly, with the consequence that in the immense majority of cases, the insects attains the limits of life, before becoming a transmitting agent of the virus, contained in its organism.

That is the reason why in the climate of the plateau of the respective regions the cases of yellow fever, attaining the cities, infected by Stegomyia and there developing themselves in their initial phase, provoking new cases, are rare, calling therefore, the attention of physicians and laymen. It is true that in past times there prevailed in the State of São Paulo and in other States of the brazilian plateau, mortiferous epidemics of yellow fever, transmitted by Stegomyia and which appeared suddenly, when summer was is its highst, in the cities, situated along the railway-roads, but it is to be noted that at these periods the indices of Stegomyia were very high and that in the dwellings the mosquitoes had at their disposition, for lack of canalized water-supply, innumerable water-vessels, reservoirs, flower-pots, etc., which offered extremely favourable conditions for the life and multiplication of Stegomyia, and, therefore it is no wonder at all that at that time the transmitting agents were very numerous. In no city of São Paulo, not even in the
smallest and less treated ones, it is now possible to find the conditions existing in Campinas, during the terrible epidemic of 1889, with its 6,500 water-wells, beside all the other private deposits in a city of more or less 15,000 inhabitants. Professor Adolpho Lutz mentions that Stegomyia were so numerous that in order to be able to read during day-time, he was obliged to shelter himself under a mosquito-net. It is, therefore, no wonder that in such conditions, in the presence of so many Stegomyia, which encountered the best living conditions within the dwellings at the most favourable temperature, the disease could break out, in very hot summers, with great violence, provoking the famous epidemics of Campinas, Sorocaba, Ribeirão Preto, Dous Corregos, S. Simão, Rio Claro, etc.

In spite, however, of the incomparably less number of Stegomyia in the cities of the plateau and of the adverse climatic conditions, there existing with respect to the transmission of yellow fever by this mosquito infected in patients within the localities the disease has already appeared here and there, giving cause to some urban epidemics, what demonstrates the possibility of passage of the jungle disease to the city. For instance, at Cambará, Northern Paraná, where a jungle form prevailed, Walcott had the opportunity of observing two dozens of cases of yellow fever in inhabitants of the city, who did not frequent the woods, and lived in a zone where Stegomyia existed. In Presidente Wenceslau, State São Paulo, in April of 1937, when the index of Stegomyia of the city was more or less 22%, Dr. Idt Pontes, chief of the Local Service of Yellow Fever, observed also two small urban foci, one of which irradiating itself in the classic way from a boarding-house, frequented at that time by several patients in the infecting phase of the disease coming from the near woods. Contrasting with the difficulty with which jungle yellow fever penetrates into the cities of the Plateau, chiefly on account of its climatic conditions, we note the facility with which it penetrates from the woods into the cities in hot and low zones, where Stegomyia has a perfectly normal vitality and where, therefore, the virus multiplicates itself very rapidly in that insect. It was that what occurred at Figueira, a locality situated at less than 200 meters of altitude in the valley of Rio Doce, and also at Theophilo Ottoni, a city of low altitude and of very hot climate with many Stegomyia, where there happened a sudden outbreak of some 100 cases of yellow fever, very probably of jungle origin, since, later on, some cases of this disease were discovered in the woods.

These instances confirm still more that the strongest barrier against penetration of yellow fever into the cities of the Plateau is its mild cli-
mate with great daily oscillations from 12° to 20° with an average of 22° and 24° even in summer. These facts prove that such places may conserve themselves immune with relatively high index of Stegomyia in view of the obstacles against infection by Aedes aegypti, but we do not believe that, notwithstanding, there may be allowed in the localities of the interior a high index of Stegomyia especially during summer, when the temperature maintains itself many times high and constant. While on the plateau Stegomyia finds very precarious life-conditions and shows itself so refractory to transmission of the jungle virus, the jungle mosquitoes, on the contrary, transmit the disease, with extreme facility, within the woods, to people who is going to work there in conditions of temperature, very inferior to those with which normally Stegomyia meets, since, as far as our observations go in this respect, the temperature oscillates more or less between 14 and 26° in the warm months, with an average of more or less 20°. It is, however, to be noted that at 20° or a little more the jungle mosquitoes meet with the same favorable life-conditions as Stegomyia at 27° to 32°, since the jungle insects have adapted themselves at living at much lower temperature than the urban mosquito. In its turn, the virus multiplies itself very well in them at a temperature which would already be improper for its evolution in Stegomyia.

From this fact there results the very interesting deduction that the multiplication of the virus of yellow fever in the mosquito has no connexion with temperature and depends, above all, upon the more or less adaptation of the insect to a given thermal level. For instance, in order to transmit well the amarillo virus, the Stegomyia wants a temperature of more than 25°, while to the jungle mosquito the temperature of 20° offers already favorable conditions for transmission. Thus the amarillo virus develops itself in the mosquito more or less as it happens in the cultures of this virus in media with living tissue, in which the virus increases and diminishes according to the cellular vitality of the culture; if that vitality is perfect, the virus develops itself very well, but, if the vitality diminishes on account of the medium becoming aged or by other reasons, the culture retrocedes, even when the temperature of the incubator remains normal, and the virus shows itself immediately less active.
III

THE PROCESS OF HUMAN INFECTION BY THE VIRUS OF YELLOW FEVER, PROPAGATED BY JUNGLE MOSQUITO.

A fact, constantly observed by us and by those who have with us, captured mosquitoes in the forest during day-time, is that the insects which then attack us, are always young specimens with perfect scales and without the slightest sign of having already sucked blood. Mosquitoes which have already once bitten, what is revealed by the ruined and already dropped scales and by the abdomen with developed eggs, do not attack spontaneously during day-time, remaining in the forest at humid places under the leaves, perfectly quiet, not going to bite again unless night, as we had the opportunity of observing at the Station of Sylvestral Biology, at Perús, São Paulo, for the species *Lutzia brasiliæ*, *Aedes leucocelanus*, *Mansonia titelans*, *Goldia pallidibenter*, etc. It is known also that the Anophelinae and the *Aedes aegypti*, which bite during day-time, are always young specimens which have not yet sucked, and therefore there is no transmission of malaria, nor of yellow fever during day-time.

After having sucked once, these mosquitoes change their habits, sucking now only at night, and, therefore, nobody acquires through the bite of anophelinae malaria or yellow fever through the bite of the Stegomyia during day-time. The same happens with the jungle mosquitoes, and only the young ones suck our blood during day-time, acquiring, however, after the first pasture, nocturnal habits. Jungle yellow fever may, however, be acquired in the woods during day-time, what happens frequently to people who stayed in the forest only during day-time, as for instance labourers, travellers, hunters, etc., and therefore it is necessary to find an explanation for that occurrence, which is so different from what we know in respect of urban yellow fever and malaria. In view of the habits of jungle mosquitoes being similar to those of the other mosquitoes, it seems to us that the cases of amarillic infection got during day-time in the woods, are not originated by the bite of mosquito, but by the entrance of the human skin in close contact with virus expelled by infected insects with fecal droplets, which, as it has been shown by our experiences made in collaboration with Dr. Costa Lima, they contain the virus which traverses easily the intact skin. This fact would explain why people, who had entered the woods only for very short time during day-time, acquired yellow fever without any probability of having been bitten by infected mosquitoes.
As the mosquitoes which have already sucked blood, expell constantly fecal droplets, it is possible that these droplets fall on the skin of people in the woods, and, if containing virus, provoke amarillic infection. Be it so this is the only way of explaining infections occurred in the woods during day-time, in people who stayed there sometimes even for an extraordinarily short period. Such people had surely not been bitten by infected mosquitoes during day-time, since during the day, people is only attacked by young mosquitoes, which have not yet sucked, as has been demonstrated by our observations, now largely confirmed. In the laboratory the feces of infected mosquitoes, deposited on the walls of the cages, and of the bottles within which the insects are conserved, remain virulent for pretty long time at room-temperature, and in the woods this conservation of virulence is, of course, still easier in view of the lower temperature and of less luminosity of the ambient.

If in the monkey the infection by simple deposition of feces of infected mosquitoes on the intact skin of the animal reproduces yellow fever, the more easier the infection will be in man, whose skin is thinner and nearly always a little moist in the forest, on account of transpiration and of the difficulty of evaporation in its an ambient, saturated by evaporation of water. Besides, it may happen in the woods that other insects, flies, tabanids, etc., while resting on a leaf containing feces of infected mosquitoes, carry the virus on its legs, and on the proboscis, depositing it, subsequently, on the skin of man.

In face of this constation, today pretty certain in view of the great number of observations that the mosquitoes which suck our blood in the woods during day-time, are always young ones which suck blood for the first time, several hypotheses present themselves. Thus it seems that in the woods a double process of transmission of yellow fever takes place, viz: during day-time without the bite of the mosquito, the human skin coming into contact which contaminated feces, and at night not only by that way, but also by the bite of mosquitoes, infected with yellow fever. The problem, presents, as we see, the most interesting epidemiological aspects which challenge the capacity of the researchers.

CONCLUSION.

All these factors make us to believe that urban yellow fever and also the rural one of North-Eastern Brazil are exclusively transmitted by Stegomyia, as proved by repeated observations of absence of such propagation to near woods in places where they prevail.

Jungle yellow fever must have been originated in consequence of
the adaptation of the urban virus to the jungle mosquitoes at a time not very distant in our opinion, since the references to the disease only appeared recently, although in other times its discovery would have been very easy in view of the existence of abundant woods and of many people working there in a large area comprising great part of 3 States of Southern Brazil, and of in view of the urban outbreaks in the cities and localities of these zones which maintained an intense human intercourse with the rural zones.

The absence of immunity against yellow fever on the part of the inhabitants of the jungle and of rural zones of the States of Southern Brazil, and the fact of no great mortality of howling monkeys (Aequatorialis) having been signalized, speak also very strong against the existence there of jungle yellow fever, in other times.

For being adapted to live at much lower temperature than Aedes aegypti, the jungle mosquitoes are capable of transmitting amarillic virus in lower thermic ambient conditions than those at which Stegomyia would be capable to do it. Therefore cases of jungle yellow fever have been observed in forest in which the conditions of ambient temperature became improper to eventual existence of the disease, when transmitted by Aedes aegypti.

The transmission of amarillic virus depends more on the adaptation of the mosquito to living at a given temperature than on a determinated degree of thermic optimum for the evolution of the yellow fever virus in it.

The jungle mosquitoes which bite during day-time are always young insects, which have not yet sucked blood; the same happens with Aedes aegypti and with the Anopheline which attack man during day-time, and for that reason neither malaria nor yellow fever, transmitted by these mosquitoes, are acquired by man before sunset.

The jungle yellow fever is commonly acquired during day-time, but that transmission is only possible, when the human skin enters into contact with the virus which the mosquitoes have deposited, with their feces, on the plants of the woods, where they repose or are sheltered.