LOCALIZED PERMANENT EPIDEMICS : THE GENESIS OF CHLOROQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM*

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Abstract. Localized permanent epidemics occur when, for an indefinite period of time, there is a temporary but continuous introduction of unprotected non-immunes into the same locality of a hyperendemic area. The main epidemiological factors involved in the genesis of localized permanent epidemics were encountered in Pailin (Cambodia) the epicenter of drug resistance in Southeast Asia:

- a very efficient vector, *Anopheles dirus*, exophilic and of limited distribution with, therefore, adjacent hyperendemic and non-endemic areas;

- a permanent pole of attraction in the hyperendemic area: Pailin's sapphires and rubies;

- a temporary but continuous influx of non-immunes into the pole of attraction: continuous influx of non-immunes into the Pailin gem mining area.

In the gem-mining Pailin village drug pressure was considerable : mass drug administration, a medicated salt project and permanent self-medication with very high doses, much higher doses being required to cure non-immunes with heavy infections and severe clinical attacks in epidemic situations. It appears, therefore, that the emergence of chloroquine resistance in Southeast Asia was the consequence of the localized permanent epidemics in Païlin. High level resistance was the result of continuous and intensive serial passages of *P. falciparum* in the non-immune subjects, large numbers of parasites being exposed to a high level of drug pressure at each passage.

Similar epidemiological conditions are encountered in some parts of South America where the exophilic vector is *An. nuneztovari*. In Colombia, whose eastern mountains bordering Venezuela yield the most highly prized emeralds in the world, chloroquine resistance was detected at about the same time as in Southeast Asia. On the other hand, epidemiological conditions are quite different in Africa, south of the Sahara, where chloroquine resistance was most probably imported from Southeast Asia.

Localized epidemics occur when groups of non-immunes, without suitable protection, move into an area of hyperendemic malaria. Such outbreaks are rather sharply circumscribed and do not tend at all to recur periodically (Russell, 1952; Pampana, 1963).

Exceptionally, there are also localized epidemics which are sharply circumscribed but tend to persist indefinitely. They might be called localized permanent epidemics and occur when, for an indefinite period of time, there is a temporary but continuous introduction of successive groups of unprotected non-immunes into the same locality of a hyperendemic area.

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THE CONCEPT OF LOCALIZED PERMANENT EPIDEMICS

Localized epidemics

The introduction of a group of unprotected non-immunes into an hyperendemic area will obviously result in an epidemic amongst the non-immunes. It may also increase transmission amongst the semi-immune local residents.

The epidemic amongst the non-immunes may be represented by Fig 1 showing the incidence of new infections. The curve is diagrammatic illustrating the chain of events following the introduction of the group at zero day, indicated by an arrow. The one-week interval following the zero corresponds to the minimum incubation period of *P. falciparum*.
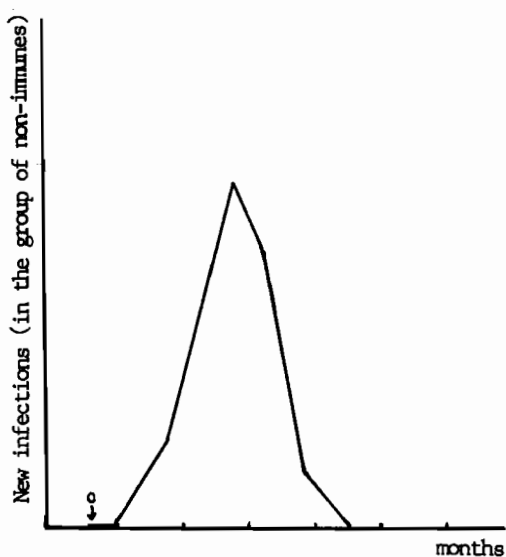
Fig 1–Localized epidemic

Fig 2–Localized permanent epidemics

The time scale and the height the curve rises depend on the value of several variables: reproduction rate, importance of the infective gametocyte reservoir, etc (Macdonald, 1957).

Localized permanent epidemics

The temporary but continuous introduction of successive groups of unprotected non-immunes into the same locality of an hyperendemic area will result in an uninterrupted series of epidemics (Fig 2).

The genesis of such localized permanent epidemics will require two basic conditions:

a) a very efficient vector, exophilic therefore not responding to residual spraying and - this is of capital importance - of selective distribution, *ie* limited to a type of epidemiological landscape with a non-endemic area and an hyperendemic area (Yin and Yang epidemiology : Fig 3).

b) a permanent pole of attraction in the hyperendemic area.

The introduction of a group of non-immunes into the hyper-endemic area will result in an epidemic. The temporary but continuous introduction of successive groups of non-immunes into the same locality of the hyperendemic area (pole of attraction) will result in an uninterrupted series of epidemics, *ie* a state of localized permanent epidemics.
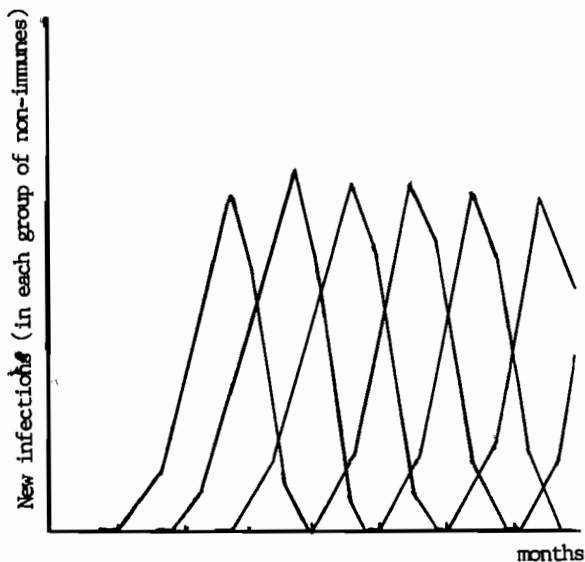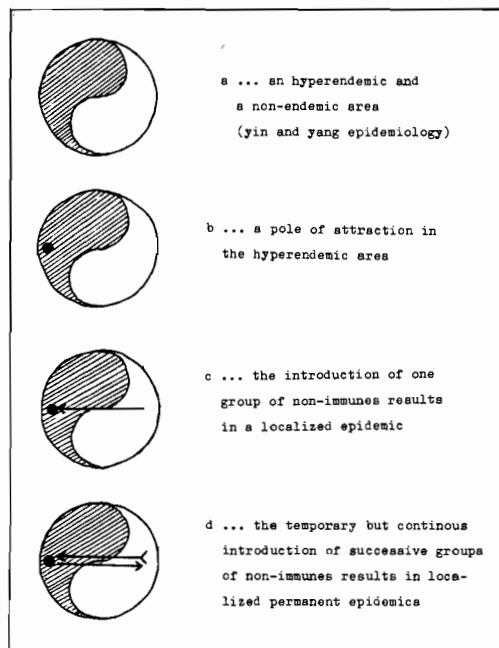

Fig 3–The genesis of localized permanent epidemics

If these events are going on for years, each successive group of non-immunes staying in the hyperendemic area for a period of 3 to 4 months, then the epidemiologic results will be most dramatic, especially if the prevalent species is *Plasmodium*

falciparum. Because of the absence of immunity in the temporary migrants, hyperparasitemia (asexual and sexual) and cerebral malaria will be common, with high morbidity and mortality rates. This state of permanent epidemics may last for 5, 10 or more years in the absence of an effective malaria control program.

This is not simply an hypothesis and, in fact, there is an excellent example to illustrate these happenings: Pailin in Cambodia.

PAILIN : A TYPICAL EXAMPLE OF LOCALIZED PERMANENT EPIDEMICS

The small town of Pailin is located on the Thai-Cambodian border. The terrain is of a hilly type, covered with dense forest and jungle. Malaria is hyperendemic. The prevalent species is *P. falci-parum* (80%).

Pailin's very efficient exophilic vector : *Anopheles dirus*

In Cambodia, as in other parts of mainland Southeast Asia, only *An. dirus* (originally known as *An. balabacensis*) has the characteristics of longevity, anthropophilism, exophily and exophagy which enable it to maintain transmission of stable malaria even under DDT residual spraying (Slooff and Verdrager, 1972). This species has a limited distribution. It is a jungle vector associated with dense shading vegetation and is not seen in developed agricultural areas. The effects of malaria on people resemble those in Africa but - and this is of capital importance - this picture prevails only in areas actually covered by dense forest and jungle. On the other hand, the densely populated fertile central plain along the Tonle Sap big lake and the Mekong river, including the capital Phnom Penh, is practically malaria free and its population non-immune (Fig 4).
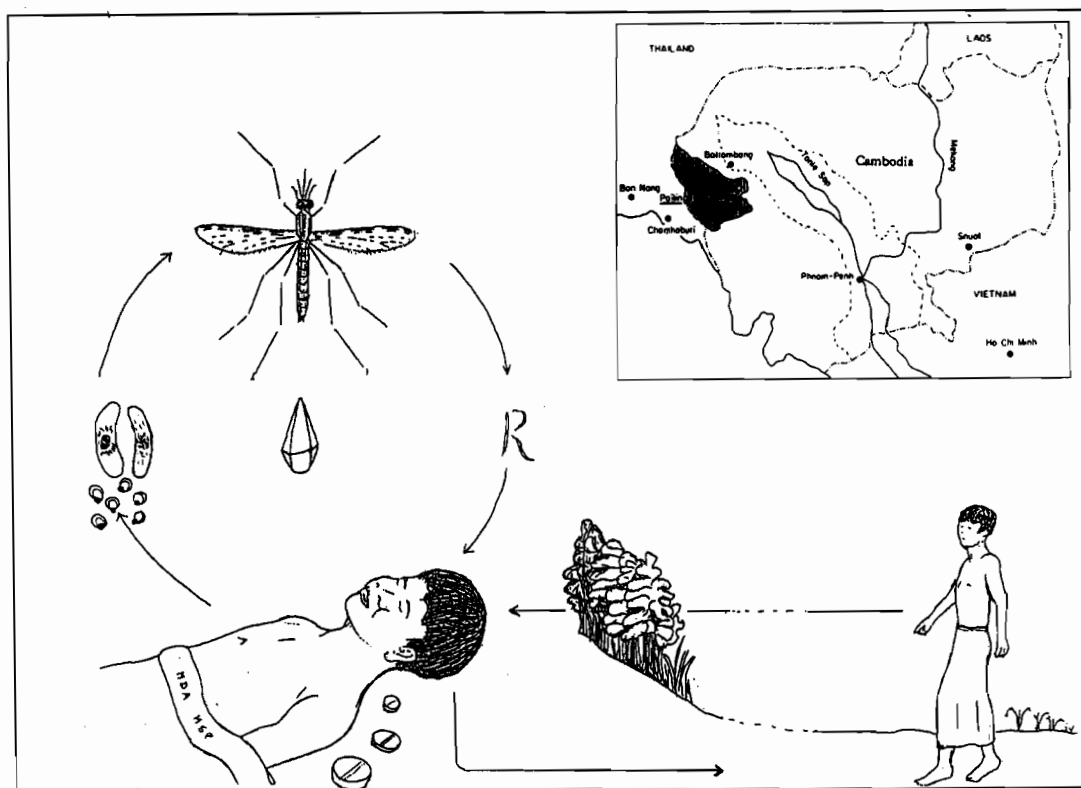

Fig 4–The genesis of drug resistance in Pailin

There is an intermediate hypoendemic area where *An. minimus* is the vector and a coastal area, also hypoendemic, where *An. sundaicus* is the vector.

A permanent pole of attraction : Pailin's blue sapphires and rubies

According to Prince Norodom Sihanouk (1981) a few hundred Burmese had already settled in Battambang Province (which includes Pailin district) in 1884 attracted by the famous blue sapphires and rubies of Pailin.

However, it is apparently only in the late 1940s or early 1950s, following the return of this province to Cambodia - territory temporarily occupied by the Thais from 1941 to 1946 - that Pailin became a permanent pole of attraction for temporary gem-mining laborers: Cambodians, Vietnamese, Chinese, Burmese and Thais.

A continuous influx of non-immunes into the hyperendemic Pailin area

The temporary migrants, some from other endemic areas, most of them (at least 80%) non-immune young adults from the central plain and the capital Phnom Penh, arrived daily in small groups of 30 to 40 (approximately 1,000 to 1,200 per month) and usually spent an average of 3 to 4 months in Phum-Thmey the "gemstone miners' Pailin village" (in French: Le village des chercheurs de pierres précieuses de Païlin), located on the outskirts of Pailin.

In this "village" migrants were most exposed because they lived mostly in the open air and slept under very rudimentary shelters. Moreover there was an increase in one of the favorite breeding places of *An. dirus:* the gem mining pits filled with water from seepages and rain.

Malaria transmission, already intense in this hyperendemic area, became *maximized* in the gem miners' village due to the increased infective gametocyte reservoir, increased number of vectors and increased contact between man and vector.

Severe and complicated malaria attacks were most common in those non-immune young adults. Deaths from cerebral malaria occurred every day. In other words, the continuous daily influx of non-immune temporary migrants into the Pailin hyper-

endemic area resulted in a state of localized permanent epidemics, an uninterrupted series of deadly epidemics which apparently started in the late 1940s or early 1950s (or much earlier but on a smaller scale). Such epidemics are still going on after more than forty years (Valette, 1993).

LOCALIZED PERMANENT EPIDEMICS AND EMERGENCE OF CHLOROQUINE RESISTANCE

The effectiveness of antimalarial drugs is undoubtedly influenced by the immune status of the patient, much higher doses being required to cure populations with heavy infections and severe clinical attacks in epidemic situations, the highest doses being required to relieve the non-imune subjects. This was particularly evident in Pailin village where very large doses and multiple drugs were used (Verdrager, 1986). Moreover, in this village, as in the whole Pailin district (Fig 4), mass drug administrations with pyrimethamine and chloroquine were carried out from 1955 to 1962:
- twice a year from 1955 to 1957
- weekly in 1958 - 1959
- daily, indirectly through medicated salt, from 1960 to 1962.

Experimental production of chloroquine resistance

Two main techniques, involving serial passages of the parasites, are used in the *in vivo* experimental production of drug resistant lines of *Plasmodium* in non-immune animals:

a) those involving exposure to constant or progressive doses in successive passages,

b) a relapse technique in which parasites are exposed to single or multiple high doses at each relapse in animals with a high infection rate (this favors selection of mutants). Latent infections (low infection rate) are unfavorable for the production of resistance (Peters and Richards, 1984).

Field production of chloroquine resistance

Similar procedures, involving intensive serial passages of *P. falciparum* under considerable drug pressure, were involved in the gem mining Pailin

village through:

- the selection of decreasingly sensitive *P. falciparum* being promoted by the impact of intermittent or constant but still inadequate subcurative concentrations of chloroquine in the blood (mass drug administration, medicated salt) that eliminated only the most sensitive parasites. (This does not apply to pyrimethamine resistance which develops very rapidly during mass-drug administration).

- the selection of high degree chloroquine-resistant *P. falciparum* being promoted by the repeated and very high multiple doses (self-medication) taken by the non-immunes during primary attacks, recrudescences or reinfections (Fig 4). In other words, emergence of high level resistance was the result of continuous and intensive serial passages of *P. falciparum* in the non-immunes, very large number of parasites being exposed to multiple high doses of the drug at each passage.

Moreover, it appears that *An. dirus* has a biological advantage in relation to the transmission of chloro-quine-resistant *P. falciparum*. Ramkaran and Peters (1969) have shown that continuing chloroquine pressure can lead to an increase in vectorial capacity. Subsequently chloroquine was shown to enhance infection of chloroquine-resistant *P. falciparum* in *An. dirus* (then *An. balabacensis*) but not in *An. minimus* (Wilkinson *et al*, 1976).

In the other parts of the hyperendemic Pailin district area, subcurative doses of chloroquine (mass drug administration and the medicated salt project) in the semi-immune population probably resulted only in a low degree of resistance (loss of sensitivity to 300 or 600 mg chloroquine base).

Multidrug resistance

In 1962 multidrug resistance to chloroquine, mepacrine, pyrimethamine and proguanil was confirmed in the Pailin area (Eyles *et al*, 1963).

In 1967 resistance to the combination sulfadoxine-pyrimethamine (Ro 6-4793, later commercialized as Fansidar®) was reported from Pailin and confirmed in 1968 with cross-resistance to the combination DDS-pyrimethamine (Verdrager *et al*, 1969).

Resistance to mefloquine has also developed

very rapidly in this Thai-Cambodian border area where this drug is now failing to cure a large proportion of the patients (50 to 60%).

This Thai-Cambodian border area can therefore be considered as a very efficient "drug-resistance factory". It is most probable that, by the same mechanisms producing the same effects, resistance to qinghaosu derivatives will also rapidly develop once these new drugs (artesunate, artemether, etc) are introduced into this area.

Chloroquine resistance in South America

Chloroquine resistance of *P. falciparum* in South America, first reported in Colombia in 1960, at about the same time as in Southeast Asia, probably emerged in the northern part of the Amazon Basin under similar epidemiological conditions, the differences being that:

- the exophilic vector was not *An. dirus* but *An. nuneztovari,* another forest mosquito with elusive habits which maintains a high degree of transmission in the border areas of Venezuela and Colombia. In Brazil, entomological studies indicate the predominant vectorial role of predominantly exophagic and exophilic *An. darlingi* and to some extent *An. nuneztovari.*

- the gems were not sapphires and rubies but emeralds. Although the most beautiful specimens are found in Colombia (Muzo), in Brazil a large variety of other gemstones is found in addition to emeralds. Moreover there are multiple gold and other mining areas where "garimpeiros" come and go freely. The non-immune migrant population is the most affected by serious epidemics where *P. falciparum* predominates. Self-medication is a widespread phenomenon in many of these areas.

Chloroquine resistance in Africa

Epidemiologic conditions, similar to those encountered in Southeast Asia or South America, do not exist in Africa south of the Sahara. Immunity is high and malaria stable. There are no localized permanent epidemics due to the continuous introduction of non-immunes.

Many other epidemiological factors (Peters, 1969; Onori, 1984; Le Bras and Coulaud, 1986; Schapira, 1990; Wernsdorfer and Payne, 1991)

strongly suggest that the appearance of chloroquine resistance in East Africa in 1977 was the mere continuation of the westward spread of resistance from Southeast Asia through the Indian sub-continent.

As already pointed out by Peters in 1969, epidemiological conditions in Africa were not favorable for the emergence of chloroquine resistance. *P. falciparum* had the potential (Nguyen-Dinh and Trager, 1978) but not the epidemiologic conditions suitable for it to develop high level resistance to chloroquine.

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