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内 容



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# ECOLOGICAL STUDIES ON THE LUNG FLUKE, *PARAGONIMUS OHIRAI* MIYAZAKI, 1939 II. INFECTION RATES WITH *P. OHIRAI* OF SNAILS AND RODENTS COLLECTED FROM THE IBI, NAGARA AND KISO RIVERS IN THE TOKAI DISTRICT, CENTRAL JAPAN

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**Abstract:** In the area of the Ibi, Nagara and Kiso rivers in the Tokai district, central Japan, a survey for the detection of *Paragonimus ohirai* in snails and rodents was performed during the period between October 1983 and October 1985. Snails, *Assiminea parasitologica,* positive for *P. ohirai* cercaria were found in 2 of 5 study sites. The over-all infection rates were 0.41% at the sites on the Ibi and 0.45% at those on the Kiso river. Two species of rodents, *Rattus norvegicus* and *Apodemus speciosus,* were collected, the infection rate for *P. ohirai* being 50% in the former and 0% in the latter species. The results of the present study and our previous report (Matsuo and Makiya, 1985) disclosed the life cycle of *P. ohirai* in the areas along the Ibi, Nagara and Kiso rivers; *A. parasitologica* is the 1st intermediate host, *Sesarma dehaani* and *S. intermedia* are the 2nd intermediate host, and *R. norvegicus* is the final host.

## INTRODUCTION

The first report of this series revealed that 2 species of crabs, *Sesarma dehaani* and *S. intermedia,* with *Paragonimus ohirai* were widely distributed along 3 rivers, the Ibi, Nagara and Kiso, which flow into the Ise Bay at the boundary between the north-eastern part of Mie Prefecture and the western part of Aichi Prefecture in the Tokai district, central Japan (Matsuo and Makiya 1985). Metacercariae were detected in the crabs collected at 17 out of 19 study sites which are situated about 5.5 to 13.0 km up from the estuaries of the rivers. The infection rate was 2.7–100% for *S. dehaani* and 0.9–100% for *S. intermedia.*

The present report deals for the first time with the infection rate of *P. ohirai* in snails and rodents serving the 1st intermediate and final hosts, respectively, collected from the study areas along the Ibi, Nagara and Kiso rivers. Although Iwata and Nagayoshi (1985) are of the opinion that *P. ohirai* is not an independent species, the authors here adopt the current theory that *P. ohirai* is an independent species within the genus *Paragonimus.*

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## Survey Areas and Methods

The survey of the 1st intermediate host, *Assiminea parasitologica,* was carried out at 5 sites on the Ibi, Nagara and Kiso rivers, shown in Figure 1, during 2 years from October 1983. The snails collected were crushed individually after measuring the shell length. Removing the broken shell fragments, the specimens were checked for *P. ohirai* cercaria under a stereomicroscope.

The final hosts, wild rodents, were captured with live traps set on the river-banks along the waterway which are covered with communities of eulalia grass, reed and other grasses. The traps were set in the afternoon and rodents caught were gathered in the next morning. The rodents were dissected after identifying species and sexes and measuring body- and tail-length. Lungs and livers were carefully examined for *P. ohirai* worms and feces were also checked for the eggs.

## Results and Discussion

First intermediate host: Two species of snails, *A. parasitologica* and *A. japonica* were collected at 5 study sites shown in Figure 1. *Assiminea parasitologica* inhabits damp areas on river-banks covered with eularia grass and other weeds, and was collected from the surface of soil, deadwood and fallen leaves. *Assiminea japonica,* on the other hand, was mainly seen on the surface of wet soil and on stones on the bottom of the river and the lower part of reed stems, which were submerged at high tide.

The detection of *P. ohirai* cercaria was carried out for *A. parasitologica* which is known as the principal 1st intermediate host in other endemic areas of *P. ohirai* in Japan (Yokogawa *et al.* 1957; Yoshida and Miyamoto 1959; Miyamoto 1961). The results are summarized in Table 1 and Figure 1. *Paragonimus ohirai* cercariae, as shown in Figure 2, were detected in snails collected at B and E out of 5 study sites. Site B is located about 8 km up from the estuary of the Ibi River, where Route 1 crosses the river. The cercariae were found in 8 of 1,782 snails (infection rate=0.45%), where the infection rate of the 2nd intermediate host crab with *P. ohirai* metacercariae was 90% for *S. dehaani* and 88.9% for *S. intermedia* as reported in the first part of this study. Site E is situated about 6 km up from the estuary of the Kiso River, where 10 out of 2,075 snails were positive for *P. ohirai* (infection rate=0.48%). The infection rate in crabs here was 69.3–85.7% in the previous report. At the other sites, all the snails examined were negative for *P. ohirai* cercariae. In conclusion, a total of 18 out of 5,305 snails examined were positive for *P. ohirai* (infection rate=0.34%).

In addition to the dissection of snails, an experiment on the cercarial emergence from snails was carried out with those collected from Site E. The snails were individually submerged in water in small petri-dish for 1–2 hours and then *P. ohirai* cercariae emerging from the snails were counted. The cercarial emergence was detected in 8 out of 2,380 snails (emergence rate=0.34%).

Yoshida and Miyamoto (1959) investigated the 1st intermediate host snails in the wide endemic area of *P. ohirai* along the Maruyama River, Hyogo Prefecture. The natural infection rate of *P. ohirai* cercaria was 0.048% for *A. parasitologica,* but negative for *A. japonica* and the experimental infection rate was 60.8% for the former and 2.0% for the latter species. The

Table 1 Infection rate of snail, *Assiminea parasitologica,* with *Paragonimus ohirai* cercariae collected along the Ibi, Nagara and Kiso rivers in Tokai district, central Japan

| | Collection site | Date of survey | No. of snails examined | No. of snails infected (%) |
|---|---|---|---|---|
| A | Ibi River | Apr., 1984 | 190 | 0 (0) |
| B | Ibi River | Jan., Jul., 1984<br>Oct., 1985 | 1,782 | 8 (0.45) |
| C | Nagara River | Jan., Jul., 1984 | 1,105 | 0 (0) |
| D | Kiso River | Aug., 1985 | 153 | 0 (0) |
| E | Kiso River | Oct., 1983<br>Jul., Oct., 1984 | 2,075 | 10 (0.48) |
| | Total | | 5,305 | 18 (0.34) |

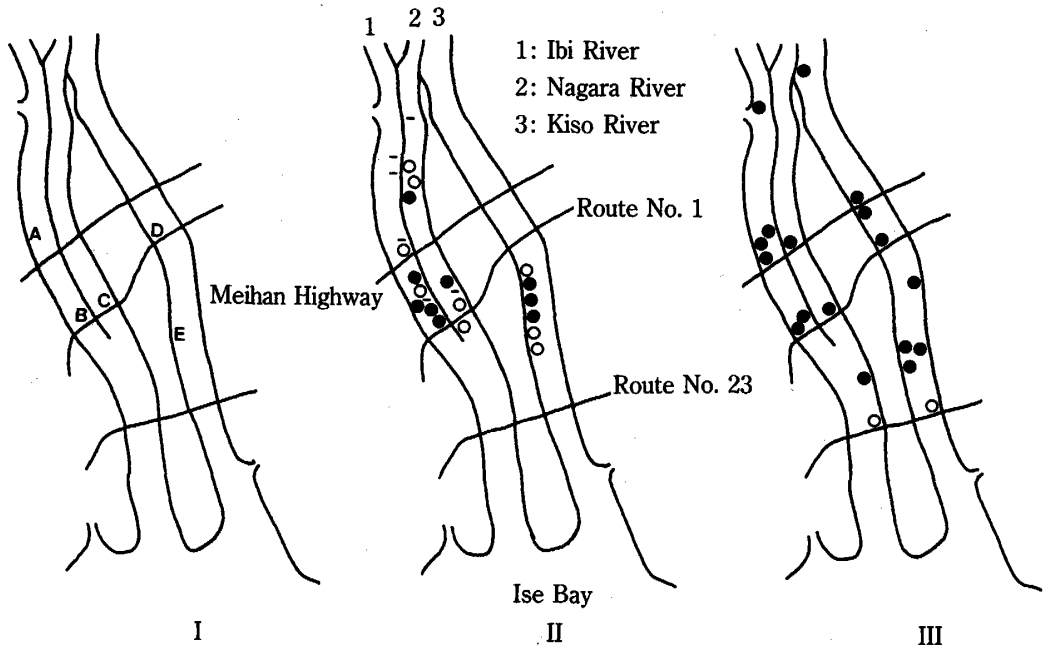
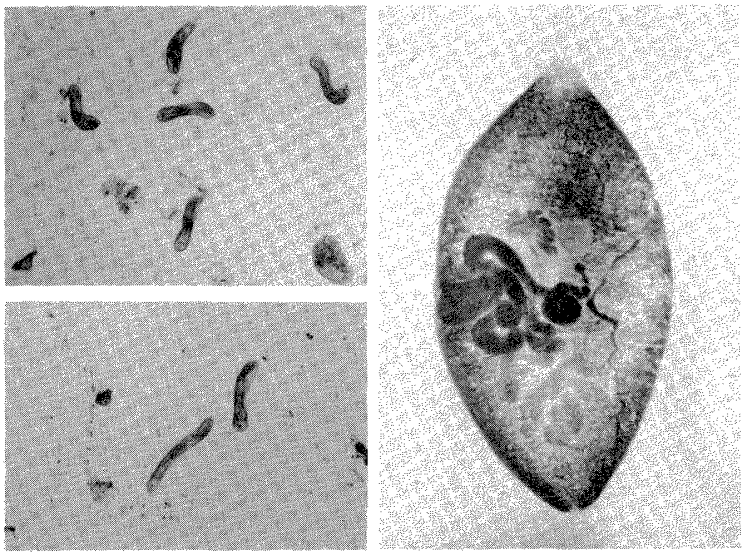



Figure 1 Sketch maps showing the Ibi, Nagara and Kiso rivers where the survey on *Paragonimus ohirai* was carried out.

I) Results of examination of the 1st intermediate host. B and E: Sites where snails positive for the cercaria were collected. A, C and D: Sites where all snails examined were negative for the cercaria

II) Results of examination of the final host. Black dots: Sites where norway rats positive for the adult worm were collected. White dots: Sites where norway rats negative for the adult worm were collected. Minus marks: Sites where wood mice negative for the adult worm were collected

III) Results of examination of the 2nd intermediate host summarized in the previous report. Black dots: Sites where crabs positive for the metacercaria were collected. White dots: Sites where all crabs collected were negative for the metacercaria



A B

Figure 2 A: *Paragonimus ohirai* cercariae recovered from the snail *Assiminea parasitologica* collected from the Ibi River. B: Adult worm of *Paragonimus ohirai* recovered from the rat *Rattus norvegicus* collected from the Nagara River.

present survey on snails revealed for the first time that *A. parasitologica* serves as the 1st intermediate host of *P. ohirai* in the areas along the Ibi and Kiso rivers and that the natural infection rate for this snail (0.45% at site B and 0.48% at site E) was 8 to 9 times higher than that in the Maruyama River area.

Final host: A total of 190 live traps were set at areas 5–12, 7–13 and 4–8 km up from the estuaries of the Ibi, Nagara and Kiso rivers, respectively, during 6 months from December 1983 to May 1984. As shown in Table 2 and Figure 1, a total of 18 norway rats (*R. norvegicus*) and 6 wood mice (*A. speciosus*) were captured during this period. Of these, 9 norway rats were positive for *P. ohirai* (infection rate=50%), but all the wood mice were negative. Eight of the infected norway rats were identified as adults, in the lungs of which adult worms of *P. ohirai* were detected. *Paragonimus ohirai* eggs were also found from the feces of all the adult

Table 2 Infection rate of rodents, *Rattus norvegicus* and *Apodemus speciosus*, with *Paragonimus ohirai* adult worms collected along the Ibi, Nagara and Kiso rivers during six month from December 1983

| Collection site | *Rattus norvegicus* | | *Apodemus speciosus* | |
|---|---|---|---|---|
| | No. examined | No. positive (%) | No. examined | No. positive (%) |
| Ibi River | 6 | 4 (66.7) | 4 | 0 |
| Nagara River | 6 | 2 (33.3) | 2 | 0 |
| Kiso River | 6* | 3 (50.0) | 0 | – |
| Total | 18 | 9 (50.0) | 6 | 0 |

* including one young rat

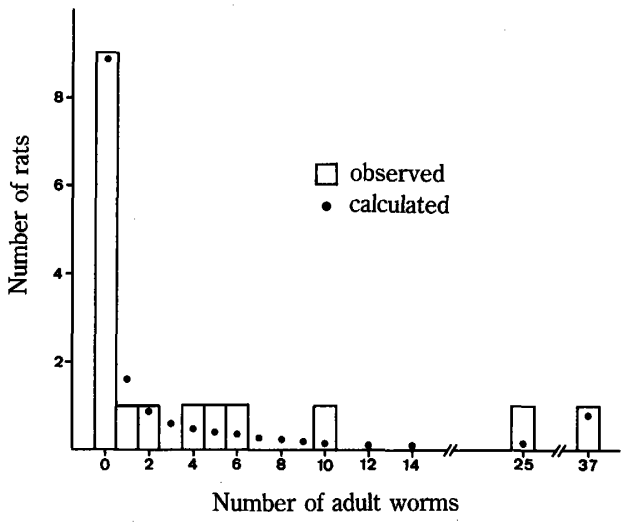



Figure 3 Frequency of *Paragonimus ohirai* adult in *Rattus norvegicus* observed frequency agreed well with the negative binomial ($\hat{k}$= 0.189, $\chi^2$=1.214, p=0.271).

rodents. One rat was young and an immature worm of *P. ohirai* was obtained from the liver of this rat. The number of worms per positive rat was 1–37, the average being 5.06. The frequency distribution of the adult worms among the final host was regarded as overdispersed, and this agreed well with the negative binomial [(Bliss and Fisher, 1953; degree of overdispersion $\hat{k}$=0.189 and fitness p=0.271 (Figure 3)]. This meant that many rats harboured a limited number of worms but that only a few individuals were infected with a large number of parasites. This type of distribution is considered to be advantageous to the parasite, because most of the parasite-carrying rats can survive longer and disseminate the eggs even if a few heavily-infected hosts may die earlier. Further, when live traps were set, crab fragments were found around the lairs of the norway rats in the area. This suggests that the crabs are one of the major food sources of norway rats in the study area.

In other endemic areas of *P. ohirai* in Japan, the natural infection rate of the final host was reported as follows: 7–35% in weasels in the Maruyama River area, Hyogo Prefecture, 47% in weasels, 25% in badgers, 14% in wild boars, 8% in Japanese meadow mice (*Microtus montebelli*) and 0% in wood mice in the Izu area, Shizuoka Prefecture.

Comparing our own with these reports, the present infection rate of norway rats (50%) is even higher than the highest rate for weasels in Shizuoka Prefecture (47%) and much higher than those of other final hosts including the Japanese meadow mouse and wood mouse. Norway rats feed not only on cereals but on fish, birds and insects, while wood mice have a mainly vegetation diet, such as the bark of young trees. It would therefore seem that the present difference in the natural infection rate between norway rats and wood mice is due to their differing food habits.

The present survey for rats revealed for the first time that the norway rat is one of the final hosts of *P. ohirai* in the areas along the 3 rivers, Ibi, Nagara and Kiso.

## Conclusions

A parasitological survey of *P. ohirai* was carried out for the 1st intermediate host in the

areas along 3 rivers, the Ibi, Nagara and Kiso in the Tokai district, central Japan. The fluke was confirmed to heavily infect the snail *A. parasitologica* and the norway rat *R. norvegicus* in the survey areas. Considered together with the previous study on the 2nd intermediate host, it was clarified for the first time that *P. ohirai* in these areas inhabits the snail *A. parasitologica* as the 1st intermediate host, the crabs *S. dehaani* and *S. intermedia* as the 2nd and the rat *R. norvegicus* as the final host.

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# 大平肺吸虫の生態学的研究

## II　東海地方の揖斐川，長良川，木曽川流域産カイならびにネズミにおける本種の検出成績

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1983年10月から1985年10月の間に，伊勢湾に注ぐ三重，愛知県下の揖斐川，長良川，木曽川の河口周辺に生息するカイ，ネズミについて大平肺吸虫の検出を行った。計5地点で採集したムシヤドリカワザンショウ計5,305個体を剖検し，そのうち，揖斐川，木曽川の各1地点から本種セルカリアを検出した。この2地点における検出率はそれぞれ，0.45%，0.48%であった。ネズミについては，3河川流域からドブネズミ計18頭，アカネズミ計6頭を捕獲して剖検した。ドブネズミでは8頭の成熟ネズミの肺から本種成虫計90個体，1頭の未成熟ネズミの肝から未熟虫体1個体を検出したが，アカネズミはすべて陰性であった。既報のカニの成績ならびに今回のカイ，ネズミの調査結果から，3河川河口流域の広大な大平肺吸虫分布地において，本種の生活史に第1中間宿主としてムシヤドリカワザンショウ，第2中間宿主としてクロベンケイ，ベンケイガニ，終宿主としてドブネズミが関与していることが初めて明らかになった。

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# LEISHMANIASIS IN DIFFERENT ALTITUDES ON ANDEAN SLOPE OF ECUADOR

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**Abstract:** An epidemiological survey was performed in a leishmaniasis-endemic area along highway which was established about 15 years ago on the Andean slope of Ecuador; the area ranged from 300 m to 1,500 m above sea level. In general survey, 64 (14.3%) of the 446 subjects examined were positive for leishmanial signs. In order to know leishmanial infections in relation to the altitudes of dwelling sites of subjects, analysis was made on 224 children with 5 to 15 years of age. At 4 different sites with 500 m, 1,000 m, 1,300 m and 1,500 m above sea level, the infection rates of the subjects from the individual sites were 17.4, 18.8, 5.6 and 8.8%, respectively. A statistically significant difference was recognized between the altitudes, 500–1,000 m and 1,300–1,500 m ($0.01<p<0.05$, $\chi^2=5.314$), but not between 500 m and 1,000 m and between 1,300 m and 1,500 m. Leishmanial infections of the children who came from forest and highway areas were compared in each altitude. But no significant difference was found between forest and highway dewellers at any study sites.

## INTRODUCTION

In Ecuador, transmission of American cutaneous and mucocutaneous leishmaniasis occurs in rural populations living in bilateral regions of the Andes mountains from the lowlands to highlands up to the elevation of 2,000 m. The disease is widely spread in most provinces and is a considerable public health problem in the country. In the endemic areas, however, little epidemiological study has been done on the community base, and no control measure has been applied to reduce or interrupt the transmission of the disease. For a future control, it would be necessary to clarify the epidemiological features in each endemic area of lowlands and highlands.

New World cutaneous or mucocutaneous leishmaniasis is more difficult to control than is those of Old World, since it is principally a disease of wild mammals in the dense forest, and numerous reservoir hosts are arboreal; thus, in most endemic areas, reservoir-vector control is almost impossible (Marinkelle, 1980). At present, the only alternative measure for the control

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in most parts of the neotropics seems to be evacuation of the entire human population from potentially dangerous areas, but such measure is inconceivable because of political, socioeconomic and logistic reasons (Marinkelle, 1980). Under such circumstance, it would be worthwhile to evaluate the effect of environmental changes in relation to the transmission of leishmaniasis.

The present paper deals with the resuslt of an epidemiological survey performed in different altitudes of leishmaniasis-endemic areas with 300 m to 1,500 m above sea level. In the area, migration of inhabitants occurred from forest area to the vicinity of highway which was constructed on the Andean slope about 15 years ago. The leishmanial infections, therefore, were also compared between forest and highway dwellers, in order to know the effect of change in the life mode of the inhabitants on the transmission.

## Materials and Methods

### *Study area*

The study site is located in the Department of Cañar on the south east of Ecuador, 2°30′ West longitude, and located on the Pacific slope of the Andes, ranging from 300 m to 1,500 m above sea level. Two villages, Ocaña and Javin, in the above area are situated about 70 km from Guayaquil City and established as agricultural communities along highway to Cuenca City. A simplified sketch of the area is shown in Figure 1.

The paved highway with 10 meters in width was constructed about 15 years ago in the area and it changed a mode of villager's life, including agricultural systems. Before construction of the highway, bananas and yucas were the main agricultural products in densely forested areas, but they were replaced by sugar canes cultivated in largely deforested areas after the highway construction. The highway supported not only the movement of villagers but also transportation of their agricultural products to major cities, such as Cuenca and Guayaquil. Thus, the highway construction made a great environmental change of the endemic area of leishmaniasis.

Still, however, there were some surviving patches of natural dense forest which would provide the breeding sites for vector sandflies and reservoir hosts of leishmaniasis. Such limited patches are distributed in the one side of the highway, while on the other side there was a continuous undisturbed dense forest through lowlands to highlands along highway (Photos. 1, 2 and 3).

The majority of dwellings in the study area were built along highway, but the remainings were in forest areas. There was no livestock, but the people raised the dogs, cats, pigs, guinea pigs and domestic fowls. Wild mammals (sloths, armadillos, opossums, rats and mice) and 2 species of man-biting sandflies, *Lutzomyia trapidoi* and *Lu. hartmanni,* were found in the area (Hashiguchi *et al.*, 1985a, b, c).

### *Epidemiological examinations*

In the inhabitants, the survey was performed by house visit, while it was done in children with 5 to 15 years of age by visiting 4 study sites (schools), La Delicia (A, 500 m above sea level), Ocaña (B, 1,000 m), Las Copas (C, 1,300 m) and Javin (D, 1,500 m). All the subjects were interviewed about their life history, such as occupation, cultivation, migration and contact history with sandflies, then examined clinically by well-experienced physician (E.A.G.L. and V.V.C.) to find ulcers (active lesions) and scars (cured lesions) of leishmaniasis. When they had active lesions, tissue samples were taken from the margin of ulcers for microscopic examina-

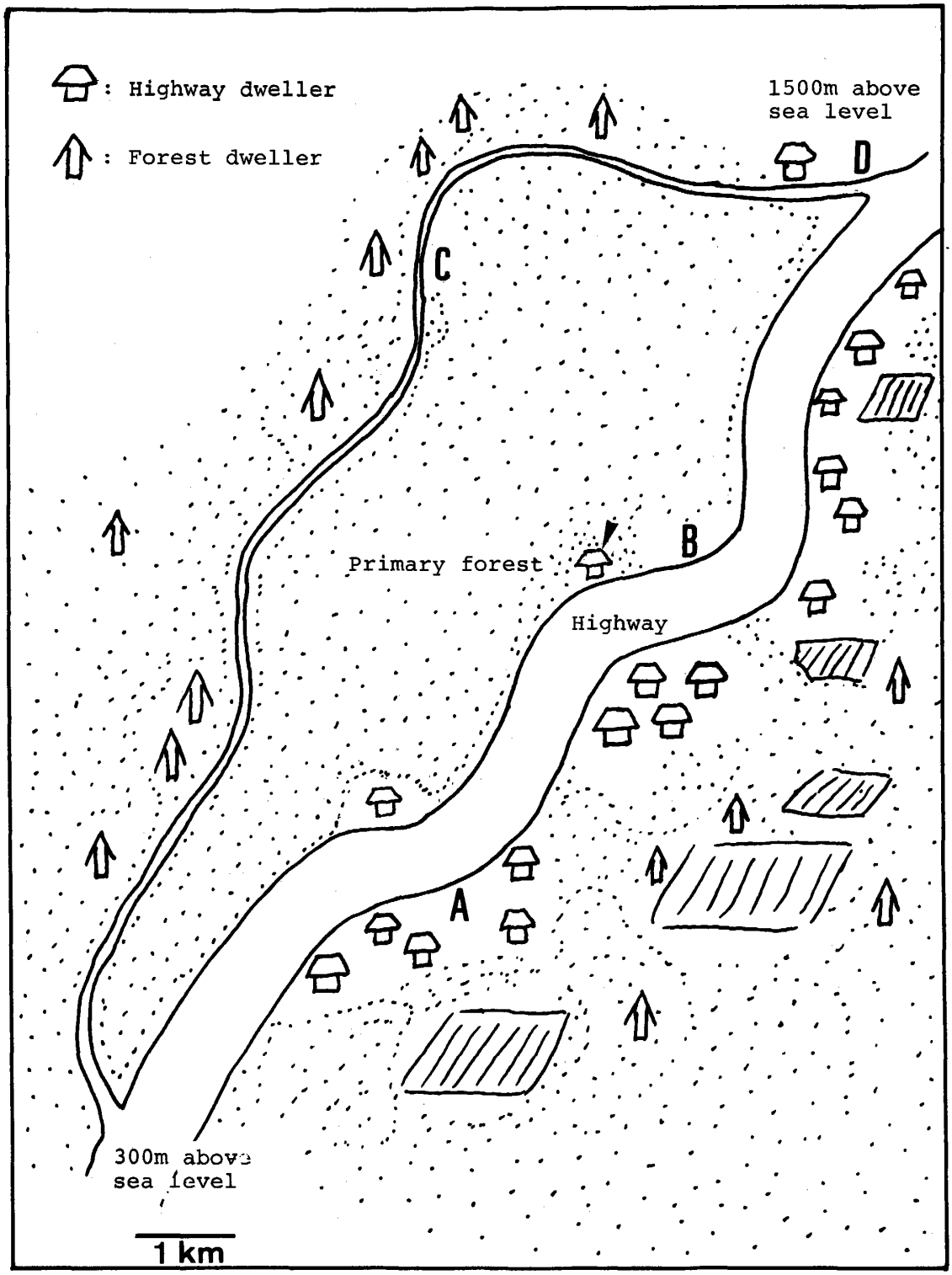


Figure 1 A simplified sketch of the study sites in the Department of Cañar, Ecuador. A, La Delicia (500 m above sea level); B, Ocaña (1,000 m); C, Las Copas (1,300 m); D, Javin (1,500 m).

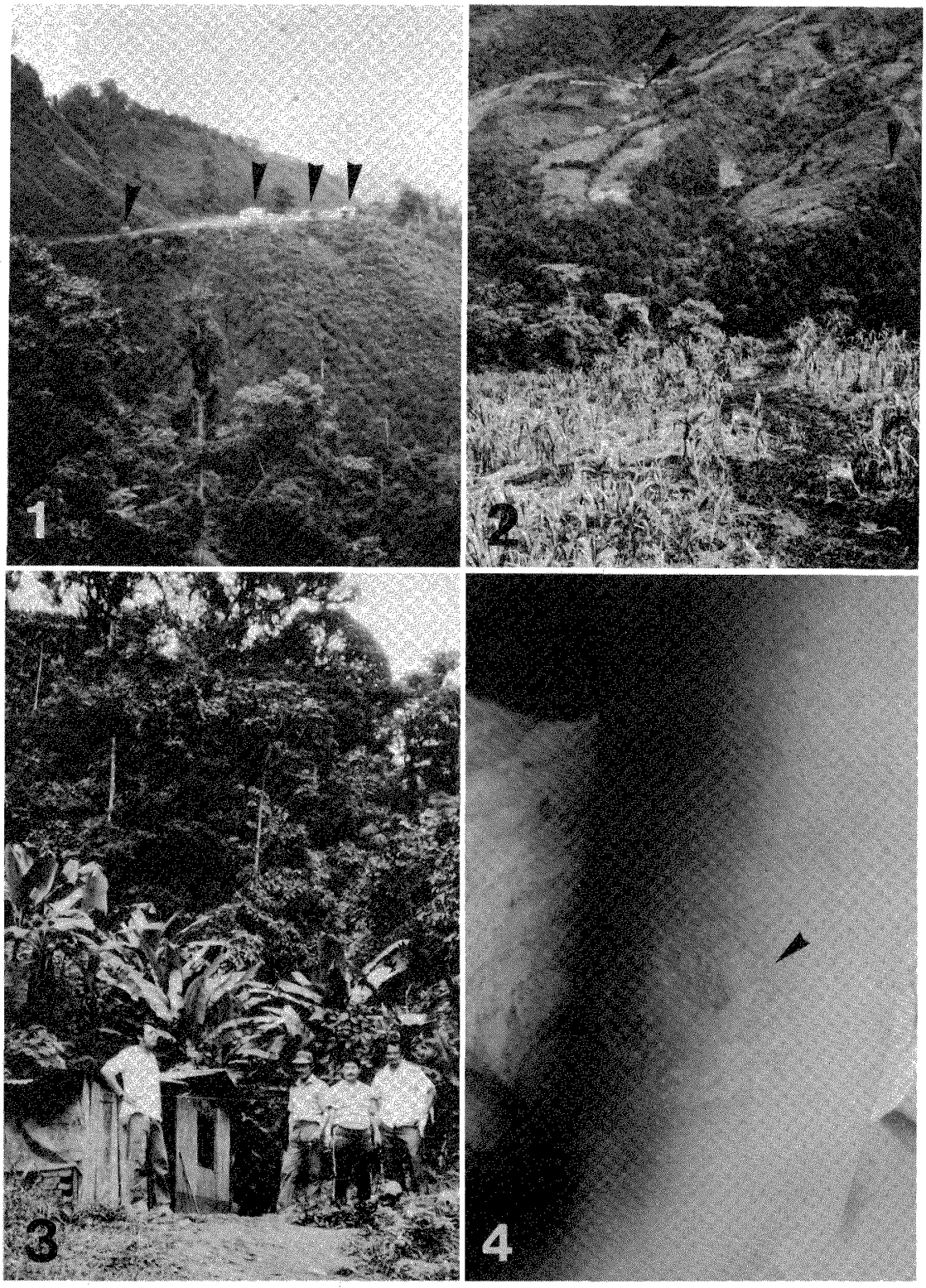


Photo. 1 Showing 4 houses (arrows) along highway in the study site (highway dwellers).
Photo. 2 Showing fields of sugar cane cultivation and remaining patches of natural (primary) forest. Arrows on the above side show 3 houses in forest area (forest dwellers).

tions. The location, size, onset and duration of lesions were recorded.

With regard to the subjects with scars, the diagnosis was determined only clinically, i.e., mainly based on the type and localization of the scars, duration of the lesions and contact history with sandflies; the chronic leishmanial ulcers resulted in a thin, depigmented scar (Photo. 4). The villagers have been calling leishmaniasis as *llaga de montaña* and sandflies, *manta blanca* in Spanish, respectively.

The causative agent of these cutaneous and mucocutaneous leishmaniasis in Ecuador has been considered *Leishmania braziliensis* s.l., based mainly on the clinical manifestations, behavior and localization of the organisms in sandfly vectors and growth in cultures.

## Results

In the inhabitants from whole area, the positive rates with leishmanial signs were arranged by age and sex in Table 1. In a total of 446 subjects examined, 64 (14.3%) were found to be positive for leishmanial signs. No marked difference was recognized between both sexes in the positive rates.

In Table 2, leishmanial infections of 224 children with age 5 to 15 years in the total examinees were arranged by the altitudes of their dwelling sites. The data revealed a statistically significant difference between altitudes, 500–1,000 m and 1,300–1,500 m above sea level ($0.01<p<0.05$, $\chi^2=5.314$), but not between 500 m and 1,000 m and between 1,300 m and 1,500 m. The result, therefore, suggested that the intensity of transmission was markedly influenced by the altitudes of dwelling sites in the endemic area.

To evaluate the intensity of transmission, the above 224 children were reanalyzed between forest and highway areas (Table 3). They were divided into the following two groups; 1) forest dwellers who have or had experience living in forest area, that is, those who settled down or

Table 1 Leishmanial infections among 446 inhabitants arranged by age and sex in the Department of Cañar, Ecuador

| Age | Male | | | Female | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. examined | +* | % | No. examined | + | % | No. examined | + | % |
| -10 | 110 | 12(4) | 10.9 | 109 | 12(3) | 11.0 | 219 | 24(7) | 11.0 |
| 11-20 | 77 | 14(3) | 18.2 | 70 | 12(1) | 17.1 | 147 | 26(4) | 17.7 |
| 21-30 | 16 | 4(1) | 25.0 | 19 | 3 | 15.8 | 35 | 7(1) | 20.0 |
| 31- | 19 | 2 | 10.5 | 26 | 5 | 19.2 | 45 | 7 | 15.6 |
| Total | 222 | 32(8) | 14.4 | 224 | 32(4) | 14.3 | 446 | 64(12) | 14.3 |

* Positives with leishmanial scars or ulcers; the number in parentheses shows positives with active lesions (ulcers).

Photo. 3 A house constructed in the vicinity of highway (1,000 m above sea level), but surrounded by dense forest (arrow in Figure 1). A considerable number of sandfly was collected around the house. All the persons of this family, 8 in total, had already suffered from leishmaniasis, showing the typical scars.

Photo. 4 A typical leishmanial scar (arrow) found on the forearm of a 34-year-old female who lives in the house shown in Photo. 3.

Table 2 Leishmanial infections of 224 children, 5–15 years old, arranged by altitude of dwelling sites in the Department of Cañar, Ecuador

| Altitude* (in meters) | Schools** | No. examined | Positives with leishmanial signs (%)*** |
|---|---|---|---|
| 500 | A | 46 | 8 (17.4) |
| 1,000 | B | 85 | 16 (18.8) |
| 1,300 | C | 36 | 2 (5.6) |
| 1,500 | D | 57 | 5 (8.8) |

* Altitude above sea level.

** A, La Delicia; B, Ocaña; C, Las Copas; D, Javin.

*** Statistically significant difference between the altitudes, 500–1,000 m and 1,300–1,500 m ($0.01 < p < 0.05$, $\chi^2 = 5.314$), but not between 500 m and 1,000 m and between 1,300 m and 1,500 m.

Table 3 Leishmanial infections of 224 children, 5–15 years old, arranged by their dwelling sites in forest and highway areas in the Department of Cañar, Ecuador

| Children from | Schools* | No. examined | Positives with leishmanial signs (%) |
|---|---|---|---|
| Forest | A | 27 | 6 (22.2) |
| | B | 34 | 5 (14.7) |
| | C | 36 | 2 (5.6) |
| | D | 11 | 1 (9.1) |
| | Total | 108 | 14 (13.0) |
| Highway | A | 19 | 6 (31.6) |
| | B | 51 | 11 (21.6) |
| | C | 0 | – |
| | D | 46 | 4 (8.7) |
| | Total | 116 | 21 (18.1) |
| Total | | 224 | 31 (13.8) |

* A, La Delicia; B, Ocaña; C, Las Copas; D, Javin.

lived in the past in forest area, 2) highway dwellers who were born at or immigrated from non-endemic area to highway area. No statistically significant difference was recognized between forest and highway dwellers in each study site of A, B, C and D.

The localization and number of lesions were examined in 52 subjects with leishmanial scars. Of 110 scars found, 40.9% were in the face, 30.0% in the upper extremities, 26.4% in the lower extremities and 2.7% in the trunk. The majority of scars measured less than 15 mm in diameter. Only 12 persons (2.7% of the total examinees), 8 males and 4 females, had 1 to 8 ulcers in the cheek, ear and upper or lower extremities. In interviews, the duration of ulcer ranged from 1 month to 2 years; the lesions measured between 4 mm and 32 mm in diameter.

## Discussion

In our previous study in the Pacific slope of the Andes in Ecuador, 15.8% of the examinees were positive for active leishmanial lesions and 60%, for leishmanial scars (Hashiguchi *et al.*, 1984). The present study showed rather low intensity of the transmission, in the Department of Cañar, Ecuador.

In the examination at 4 sites with different altitudes, the prevalences were higher at 500 m (17.4%) and 1,000 m (18.8%) above sea level than 1,300 m (5.6%) and 1,500 m (8.8%). The fact is quite noticeable in connection with the infection of sandfly vectors with leishmanial promastigotes. In this area, Hashiguchi *et al.* (1985c) examined natural infections with the parasites in man-biting species of sandflies, and reported that the infection rate of *Lu. hartmanni* was 5.9% at 350–600 m, 3.8% at 950 m, 2.3% at 1,200–1,500 m and 0% at 2,000 m, while the other sandfly, *Lu. trapidoi,* was positive for the parasites at only one site of 350–600 m (8.1%), showing a markedly reduced number of fly catches at higher sites. These results indicated that leishmanial transmission in the Andean slope was greatly influenced by the altitudes of dwelling sites, and also that the intensity of transmission would be very low at higher lands of 1,300 m or over.

By the data analysis of 224 children, the prevalence of leishmaniasis was compared between forest and highway dwellers revealed no marked difference between two groups. The result, therefore, suggested that there might be no remarkable difference between forest and highway areas, in terms of the intensity of leishmaniasis transmission in the present endemic area. This might be due to the existence of primary forest along highway or the remaining patches of natural forest around dwellings, which would play a role as the breeding sites for the vector and reservoir of the disease. In Panama, an insular effect resulting from clearance of primary forest which surrounded a settlement, guarded properly the community against leishmanial infection prevalent in the nearby forest (Herrer *et al.*, 1976). To reduce the transmission in the present area, a further clearance of the forested areas would be necessary.

The localization of lesions in the subjects examined mostly agreed with that reported by Rodriguez and Aviles (1953) and Hashiguchi *et al.* (1984) from Pacific coastal regions of Ecuador, but greatly differed from that in Amazon regions of the country, where 60% of the lesions observed were in lower extremities (Amunarriz, 1982). This discrepancy might be caused by the difference of biting behavior of sandflies or clothing habits of inhabitants between the two regions.

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# エクアドル国アンデス斜面の高度差による リーシュマニア症浸淫

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エクアドル国のアンデス斜面低地から高地（海抜 300 m－1,500 m）にかけて居住する住民について，リーシュマニア症の罹患状況を調べた。この流行地には約15年前にハイウェイ（道幅約 10 m）が建設され，住民の居住環境ならびに生活様式に大きな変化が認められた。

一般住民446名について検査したところ，64名（14.3%）は本症によると考えられる治癒病変，または皮膚潰瘍を保有していた。

居住地の高度差による住民のリーシュマニア症罹患状況を知るため，被検者のうち5－15歳の学童224名を対象に，4地点（A, 海抜 500 m; B, 1,000 m; C, 1,300 m; D, 1,500 m）において，居住地区別の罹患を比較した。その結果，学童のリーシュマニア症罹患率は，海抜 500 m 地点で17.4%，1,000 m で18.8%，1,300 m で5.6%，1,500 m で8.8%となり，500 m－1,000 m の地域と 1,300 m－1,500 m の地域との間には，統計学的に有意の差を認めた（$0.01<p<0.05$, $\chi^2=5.314$）。このことは，アンデス斜面のリーシュマニア症流行地の比較的低い地域（1,000 m 以下）では，本症の罹患率が高くなるが，より高い地域では罹患率は低くなることを示唆している。

一方，ハイウェイ沿いと山間部との間で，本症罹患率の差異を検討するため，上記224名の学童を，その居住地の状況によって次の2群に分類した。1) 山間部に定住または過去に一時期居住した者，2) ハイウェイ沿いで出生または非流行地から移住した者。上記2群間での学童の罹患率を見る上で，高度差による影響を除去するため，各地区ごとの山間部住民とハイウェイ住民との比較を行った。その結果，4地点のいずれにおいても両群間に有意な差を認めなかった。したがって，本調査地においては，ハイウェイが建設され，環境変化や住民の移動がみられたものの，道路沿いの原生林や人家，および農耕地周辺に原生林の一部が残存し，これがサシチョウバエや保虫宿主の供給源の役割を果たしているものと判断された。

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# CHANGES OF BLOOD OXYGEN AFFINITY IN FANSIDAR-TREATED MICE INFECTED WITH *PLASMODIUM BERGHEI*

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**Abstract:** Changes of blood oxygen affinity in mice infected with *Plasmodium berghei* and treated with Fansidar (20 mg/kg body weight sulfadoxine and 1 mg/kg body weight pyrimethamine orally) were observed. When 5-weeks-old male mice were inoculated intraperitoneally with $10^7$ of *P. berghei*-infected red cells and treated with Fansidar on day 5 after inoculation, 67% of the animals survived. Parasitemia decreased and abnormal values of glycolytic intermediates began to recover following Fansidar administration. However, hemoglobin content still decreased on day 2 of the treatment, and methemoglobin fraction increased on days 1, 2, 3 and 4. Blood oxygen affinity increased markedly on days 2 and 3. These findings suggested aggravation of the host's hypoxic state on day 2 of the treatment.

## INTRODUCTION

Malaria is relatively easy to cure if treated properly on the basis of early diagnosis. However, delay in the onset of treatment, especially for falciparum malaria, causes a higher level of parasitemia, severe complications and sometimes death of the host. Furthermore, death of patients with markedly decreased parasitemia following antimalarial treatment have been reported (Devakul *et al.*, 1966; Stone *et al.*, 1972), but its mechanism is not yet understood.

The pathophysiology of falciparum malaria is considerably complex. Most organs are reported to be involved in this infection, and many factors contributing to dysfunction of these organs have been enumerated (WHO, 1980; Bruce-Chwatt, 1980). Tissue hypoxia is thought to be one of the main causes of severe complications (Hall, 1977). We have already reported that the decrease in blood oxygen affinity in the course of *Plasmodium berghei* infection in mice may be a compensatory mechanism for tissue hypoxia resulting from malaria (Ohtomo *et al.*, 1982).

In this study, we intended to clarify the effect of treatment on blood oxygen affinity in *P. berghei* infected mice. We used Fansidar as an antimalarial drug because it is effective in a single dose and has clear action of only blocking the 2 sequential stages of the pathway leading to tetrahydrofolate (Donno, 1974; Weidekamm *et al.*, 1982).

## MATERIALS AND METHODS

Male mice of ddY strain, 5 weeks old, were used in this study. *Plasmodium berghei*, strain NK65, has been maintained in our laboratory by injecting a dilute suspension of infected blood

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into mice from the corresponding strain every week. Blood was collected from an infected mouse by cardiac puncture using heparin as an anticoagulant, diluted with saline to yield $1\times10^8$ infected red cells/m*l*, and 0.1 m*l* of this suspension was injected intraperitoneally. The time interval between exsanguination and inoculation was less than 1 hour. Mice were kept at 24–26°C and 40–60% humidity and exposed to a photoperiod of 12 h light and 12 h darkness. Under these conditions, mice died 6–7 days after inoculation.

In the initial trials, designed to evaluate the time of treatment, 3 groups of 6 mice each were infected. Fansidar (F. Hoffmann-La Roche, Switzerland) was dissolved in distilled water and administered orally (1 mg pyrimethamine and 20 mg sulfadoxine/kg body weight) on day 4 after inoculation to group 1, day 5 to group 2, and day 6 to group 3, respectively (Ferraroni and Speer, 1982). The course of the infection was followed by determining parasitemia and reticulocyte count daily first by new methylene blue- followed by Giemsa-stained blood smears from the tail vein.

In the present study, the mice were inoculated with *P. berghei* and treated with Fansidar on day 5 of infection. Eleven mice each were anesthetized with pentobarbital sodium (50 mg/kg body weight intraperitoneally) on days 0, 1, 2, 3, 4, 5 or 6 after Fansidar treatment, and whole blood was collected into heparinized syringes from the carotid artery within 5 minutes. Parasitemia was determined on Giemsa-stained thin blood film. Total hemoglobin concentration was measured by the cyanmethemoglobin method and methemoglobin fraction according to the KCN addition method (International Committee for Standardization in Haematology, 1978; Zwart *et al.*, 1981). Hematocrit was determined by means of a microhematocrit centrifuge. Oxygen equilibrium curve (OEC) of whole blood was measured at 37°C on a Hem-O-Scan analyzer (Aminco Co., USA), and blood oxygen affinity was expressed as the half-saturation pressure values at actual pH *in vivo* ($P_{50}$ act pH). The Hill-coefficient *n* was obtained by linear regression analysis of log [y/(1−y)] vs. log $Po_2$, where y is $O_2$ saturation for the data points of the OEC ($O_2$ saturation=30, 35, 40, 45, 50, 55, 60, 65 and 70%). Whole blood pH was measured immediately at 37°C with a glass electrode. 2, 3-Diphosphoglycerate concentration was determined according to a modified kit (No. 148334; Boehringer Mannheim GmbH, F. R. Germany) of the enzymatic end-point method. Blood adenosine triphosphate (ATP) concentration was determined by means of the luciferase reaction with a CHEM-GLOW (Aminco Co.) (Chapman *et al.*, 1971). Concentrations of blood glucose, pyruvate and lactate were determined according to a modified kit (Nos. 124028, 124982 and 124842; Boehringer Mannheim GmbH).

## Results

Fansidar treatment on day 6 of *Plasmodium berghei* inoculation resulted in 100% mortality in mice 1–2 days after treatment. Sixty-seven percent of mice treated on day 5 and 100% of those on day 4 survived more than 10 days after treatment (Table 1). Parasitemia decreased following treatment compared with increasing in untreated subjects. Simultaneously, polychromatophilic erythrocytes in the peripheral blood began to increase and significant reticulocytosis was observed from day 2 after treatment ($p<0.05$). When Fansidar was given on day 5, most of the parasites appeared to have degenerated on day 2 of treatment, and parasitemia became undetectable on day 4. Reticulocyte count reached its peak on day 5 after treatment, although uninfected controls remained low (Table 1).

Parasitemia of mice sacrificed for measurement (Table 2) were much the same as above.

Table 1 Time course of changes in parasitemia and reticulocytosis of mice treated with Fansidar 4 and 5 days after *Plasmodium berghei* inoculation

| Day of Fansidar treatment | | Days after Fansidar treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 8 | 10 |
| 4 days after inoculation | No. of surviving mice | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Parasitemia (%) | 26.4 ±5.0 | 7.5 ±1.6 | 1.7 ±0.5 | 0 ±0 | 0 ±0 | 0 ±0 | 0 ±0 | 0 ±0 | 0 ±0 |
| | Reticulocyte (%) | 0.5 ±0.2 | 3.4 ±0.6 | 6.0 ±1.3 | 10.0 ±1.3 | 23.7 ±6.0 | 28.5 ±4.2 | 19.6 ±2.9 | 15.9 ±1.3 | 11.7 ±2.0 |
| 5 days after inoculation | No. of surviving mice | 6 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | Parasitemia (%) | 62.7 ±2.2 | 41.7 ±5.8 | 10.7 ±2.9 | 2.4 ±1.0 | 0 ±0 | 0 ±0 | 0 ±0 | 0 ±0 | 0 ±0 |
| | Reticulocyte (%) | 0.4 ±0.1 | 4.1 ±0.5 | 15.6 ±1.6 | 17.9 ±3.3 | 29.1 ±4.1 | 35.2 ±6.4 | 31.0 ±3.5 | 20.9 ±4.2 | 8.9 ±0.2 |
| Uninfected controls | No. of mice | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Reticulocyte (%) | 2.8 ±0.1 | 2.9 ±0.2 | 2.6 ±0.2 | 2.7 ±0.3 | 2.9 ±0.3 | 2.7 ±0.3 | 2.6 ±0.2 | 2.7 ±0.3 | 3.1 ±0.4 |
| Uninfected and Fansidar treated controls | No. of mice | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Reticulocyte (%) | 2.9 ±0.4 | 3.2 ±0.3 | 2.7 ±0.3 | 3.2 ±0.3 | 2.6 ±0.3 | 2.9 ±0.3 | 2.7 ±0.2 | 2.9 ±0.4 | 2.9 ±0.4 |

Values are means±SE of surviving mice.

Table 2 Hematological data of mice infected with *P. berghei* and treated with Fansidar on day 5 of infection

| | Uninfected controls | | Days after Fansidar treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No treatment | Fansidar treatment | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Parasitemia (%) | – | – | 65.5 ±2.6 | 43.7 ±5.0 | 12.6 ±1.4 | 1.5 ±0.7 | 0 ±0 | 0 ±0 | 0 ±0 |
| Hb (g/dl) | 12.8 ±0.2 | 12.7 ±0.2 | 7.2 ±0.3 | 7.4 ±0.4 | 4.6 ±0.2 | 5.7 ±0.7 | 6.5 ±0.3 | 7.6 ±0.6 | 9.9 ±0.3 |
| Ht (%) | 40.5 ±0.7 | 40.8 ±0.6 | 25.7 ±1.1 | 25.4 ±1.1 | 16.9 ±0.8 | 20.6 ±1.8 | 25.6 ±1.4 | 28.4 ±1.8 | 36.6 ±0.7 |
| MCHC (%) | 31.7 ±0.2 | 31.2 ±0.3 | 27.9 ±0.6 | 29.2 ±0.5 | 27.4 ±0.7 | 27.2 ±1.0 | 25.7 ±0.5 | 26.9 ±0.7 | 27.1 ±0.5 |

Values are means±SE for 11 sacrificed mice each. Hb, hemoglobin; Ht, hematocrit; MCHC, mean corpuscular hemoglobin concentration.

Hemoglobin content and hematocrit decreased on day 2 of treatment and then increased from day 3 as summarized in Table 2. No significant changes were seen in the parameters of the uninfected controls after the administration of Fansidar. Methemoglobin fraction elevated on days 1 (9.3±1.0%, mean±SE, n=6), 2 (13.8±3.2%), 3 (9.6±2.9%) and 4 (13.6±2.3%) after treatment compared to days 0 (5.4±0.9%), 5 (5.9±1.8%) and 6 (4.3±1.5%), and uninfected controls and uninfected-Fansidar-treated ones showed 0.8±0.1% and 0.9±0.1% respectively.

Mean corpuscular hemoglobin concentration (MCHC) remained lower from day 0 to day 6 compared to uninfected controls ($p<0.01$) and reached its lowest level on day 4. $P_{50}$ act pH, reflecting blood oxygen affinity, increased dramatically on day 1 of treatment compared to uninfected controls and day 0 ($p<0.01$). Then, it diminished on days 2 and 3 ($p<0.001$) (Table 3). Table 3 also presents the Hill's *n* value reflecting the slope of the OEC, showing an increase after treatment ($p<0.001$). Changes in red cell 2, 3-DPG and ATP concentrations and in blood pH are also shown in Table 3. Red cell ATP concentration began to increase from day 1 of treatment and 2, 3-DPG from day 2. Although blood pH continued to be decreased on day 1, it returned abruptly to within normal limits on day 2. Concentrations of glycolytic intermediates are summarized in Table 4. Blood glucose concentration, which had decreased on day 5 of infection to about one third of uninfected controls, began to increase after Fansidar treatment. In contrast, blood pyruvate and lactate concentrations increased after the infection and remained high up to day 1 of treatment but began to decrease from day 2.

Table 3 Mean values and SE (n=11) of parameters measured in mice infected with *P. berghei* and treated with Fansidar on day 5 of infection

| | Uninfected controls | | Days after Fansidar treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No treatment | Fansidar treatment | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| $P_{50}$ act pH (Torr) | 40.0 ±0.2 | 41.0 ±0.5 | 52.7 ±1.1 | 61.5 ±2.6 | 47.8 ±1.6 | 43.8 ±0.6 | 42.4 ±1.0 | 43.3 ±0.9 | 44.4 ±1.2 |
| Hill's *n* | 3.02 ±0.01 | 2.99 ±0.01 | 2.87 ±0.03 | 3.03 ±0.02 | 3.21 ±0.04 | 3.25 ±0.03 | 3.10 ±0.05 | 3.13 ±0.03 | 3.22 ±0.08 |
| 2, 3-DPG ($\mu$M/gHb) | 28.9 ±1.0 | 30.2 ±1.2 | 15.8 ±2.5 | 13.8 ±2.0 | 21.8 ±1.5 | 26.1 ±0.8 | 23.9 ±1.2 | 22.2 ±1.0 | 23.1 ±1.0 |
| ATP ($\mu$M/gHb) | 5.8 ±0.1 | 5.7 ±0.1 | 4.9 ±0.1 | 6.4 ±0.3 | 8.2 ±0.4 | 9.3 ±0.7 | 10.6 ±0.4 | 9.4 ±0.5 | 9.0 ±0.3 |
| Blood pH | 7.43 ±0.01 | 7.42 ±0.01 | 7.06 ±0.04 | 7.08 ±0.06 | 7.39 ±0.01 | 7.41 ±0.01 | 7.41 ±0.01 | 7.41 ±0.01 | 7.43 ±0.01 |

$P_{50}$ act pH, partial pressure of oxygen at 50% oxyhemoglobin saturation (actual pH, temp=37°C); 2, 3-DPG, 2, 3-diphosphoglycerate. Hill's *n* values are calculated by linear regression analysis of log [y/(1−y)] vs. log $Po_2$, where y is $O_2$ saturation.

Table 4 Concentrations of glycolytic intermediates before and after treatment of *P. berghei*-infected mice with Fansidar on day 5 of infection

| | Uninfected controls | | Days after Fansidar treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No treatment | Fansidar treatment | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Glucose (mM/*l*) | 12.1 ±0.5 | 12.9 ±0.4 | 3.6 ±0.4 | 5.0 ±1.3 | 8.1 ±0.6 | 9.0 ±0.7 | 8.9 ±0.5 | 8.1 ±0.5 | 8.7 ±0.4 |
| Pyruvate (mM/*l*) | 0.11 ±0.01 | 0.09 ±0.01 | 0.34 ±0.02 | 0.46 ±0.04 | 0.24 ±0.04 | 0.14 ±0.03 | 0.13 ±0.01 | 0.12 ±0.02 | 0.13 ±0.02 |
| Lactate (mM/*l*) | 3.4 ±0.3 | 4.0 ±0.2 | 10.0 ±0.4 | 12.1 ±0.8 | 8.3 ±0.4 | 6.3 ±0.9 | 7.2 ±1.3 | 5.9 ±1.8 | 4.8 ±0.4 |

Values are means±SE for 6 sacrificed mice each.

## Discussion

*Plasmodium falciparum* causes severe infection in humans, because it invades red cells of all ages and multiplies rapidly. If patients are not provided with rapid diagnosis and treatment, they may expire due to severe complications.

We have studied the pathophysiological significance of hypoxia in malaria using experimental animal models. The present study was designed to clarify more accurately the mechanism leading to death when treatment is delayed. We investigated blood oxygen transport in mice treated with Fansidar on day 5 after *P. berghei* inoculation, and 67% of the mice treated on day 5 survived compared with a 100% survival rate for those treated on day 4 in the initial study.

Hemoglobin concentration decreased on day 2 of Fansidar treatment although parasitemia decreased following treatment. Such a reduction in hemoglobin concentration after treatment against acute malarial infection has been widely accepted (Devakul *et al.*, 1966; Abdalla *et al.*, 1980). To make matters worse, in this situation, methemoglobin fraction increased after Fansidar treatment. It is suggested that the oxygen supply to the tissues decreased due to the presence of methemoglobin in the red cells which causes not only a loss of oxygen binding capacity in the blood but also a decrease in oxygen unloading from the bood (Darling and Roughton, 1942). $P_{50}$ act pH markedly increased on day 1 of treatment and began to decrease from day 2, although it remained higher compared to uninfected controls. Such a decrease of affinity is an unfavorable change in the blood oxygen supply. In contrast, Hill's *n*, reflecting the slope of the OEC, increased after treatment. This change appears to be somewhat advantageous to blood oxygen transport. Considerisng the available hemoglobin content, however, the blood oxygen-supplying capacity seems to be lowest on day 2 of treatment. It is suggested that this might be one explanation for sudden death of falciparum malaria patients after parasitemia has decreased markedly following chemotherapy (Devakul *et al.*, 1966; Stone *et al.*, 1972).

Reticulocyte count began to increase following treatment. This may be a reaction of the body after successful treatment of an anemic situation, and also be due to the decrease in *P. berghei* which preferentially invades reticulocytes (Büngener, 1979). An increase in hemoglobin content followed this compensatory reticulocytosis, and anemia gradually vanished. A rapid increase in red cell 2, 3-DPG concentration after treatment may be due to a higher concentration of 2, 3-DPG in the increased younger erythrocytes (Mairbäurl *et al.*, 1983).

Blood glucose concentration increased from day 1 of treatment, possibly resulting from inactivated glycolysis caused by the parasites subsequent to the decrease in parasitemia (Homewood, 1977; Homewood and Neame, 1983). However, blood pyruvate and lactate concentrations remained high on day 1 and began to decrease from day 2. This may have been due to the parasites still present in the blood, and also partly to the inability of the host to reduce them because malaria causes reduction in renal microcirculatory flow, hypoxemia, tissue hypoxia and hepatic dysfunction (WHO, 1980; Hall, 1977). Lactate accumulation in the tissue, especially in the brain which is protected by a blood-brain barrier (Pardridge and Oldendorf, 1977), may persist longer than in the blood following treatment. Although blood lactate concentration still remained high on day 2, blood pH increased to 7.39. It is suggested that the carbonic acid-bicarbonate buffer system may play an important role in normalizing blood pH. Such an increase in pH may help to account the decrease in $P_{50}$ act pH (Bellingham *et al.*, 1971). It should be borne in mind that correction of blood pH of a malaria patient may cause tissue hypoxia by increasing blood oxygen affinity.

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# ファンシダールによるマラリア感染治療後の マウス血液酸素親和性の変動

日置　敦巳・大友　弘士

抗マラリア薬ファンシダール投与後の，マラリア感染マウスにおける血液酸素親和性の変動について調べた。5週齢，雄のddYマウスに*Plasmodium berghei* NK65感染赤血球$10^7$個を腹腔内接種し，その5日後にファンシダール（スルファドキシン20 mg/kg体重，ピリメサミン1 mg/kg体重）を経口投与したところ，33%のマウスは治療の1～2日後に死亡したが67%のマウスはファンシダール投与後7日以上生存した。これらのマウスでは治療によりparasitemiaは低下し，低血糖，続いて高乳酸血症も徐々に回復した。しかし，血液中の総ヘモグロビン濃度は治療2日後に低下し，ヘモグロビン中に占めるメトヘモグロビンの割合は逆に増加した。血液の酸素親和性は治療翌日には低下を示したが，2日後および3日後には著しく上昇した。この上昇は，主として血液pHの回復に起因するものと考えられた。酸素運搬に有効な血液中のヘモグロビン濃度と酸素親和性の変動から推察すると，治療後，特にファンシダール投与2日後の血液酸素供給能はかなり低下しているものと考えられた。

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# DECREASE TREND IN PROPORTIONS OF HEPATITIS B SURFACE ANTIGEN CARRIER IN CHRONIC HEPATITIS, CIRRHOSIS AND CIRRHOSIS WITH HEPATOCELLULAR CARCINOMA

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**Abstract:** Histopathological analysis was carried out to determine whether hepatitis B surface antigen with the autopsy diagnoses of chronic hepatitis, cirrhosis and/or hepatocellular carcinoma declined between the 1964–1973 and 1974–1983 decades. In this study, the liver specimens from 422 autopsy cases at Nagasaki University Hospital were used. All of the 17 cases of acute hepatitis were hepatitis B surface antigen negative. Hepatitis B surface antigen positive rates in chronic hepatitis and hepatocellular carcinoma showed minor changes during 1964–1983. On the other hand, proportions of hepatitis B surfce antigen carriers in cirrhosis and cirrhosis with hepatocellular carcinoma in the recent decade 1974–1983 were found to decrease compared to the last decade 1964–1973. However, the difference of the hepatitis B surface antigen positive rates in cirrhosis with hepatocellular carcinoma between the 2 periods was marginally significant, but not significant in cirrhosis. A possible explanation may be that many cases of posttransfusion hepatitis in the latter decade are not caused by hepatitis B virus.

## INTRODUCTION

The most plausible explanation for the increased risk of hepatocellular carcinoma is that the acceleration of cellular replication that occurs in cirrhosis enhances the effects of many carcinogens, including hepatitis B surface antigen. Moreover, the oncogenic potential of hepatitis B virus in the development of hepatocellular carcinoma has been reported (Sherlock *et al.*, 1970; Vogel *et al.*, 1970; Charinuvati *et al.*, 1975; Senba *et al.*, 1984, 1985).

After the discovery of the Australia antigen (hepatitis B surface antigen) by Blumberg *et al.* (1967), the subsequent demonstration by Krugman *et al.* (1967), and Prince (1968) that the Australia antigen was specifically associated with type B hepatitis, it was widely assumed that development of efficient screening tests would detect type B hepatitis infection carriers and make possible the eradication of the problem of posttransfusion type B hepatitis by preventing transfusion of hepatitis B virus positive blood. In Nagasaki University Hospital, hepatitis B surface antigen screening test has been performed since 1974. Therefore, the objective of this study is to see whether hepatitis B surface antigen positive rates in chronic hepatitis, cirrhosis, and cirrhosis with hepatocellular carcinoma have changed or not between the 1964–1973 and 1974–1983 decades.

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## Materials and Methods

The liver specimens were collected at Nagasaki University Hospital from 422 autopsy cases of various liver diseases, including acute hepatitis (17 cases), chronic hepatitis (40 cases), cirrhosis (177 cases), cirrhosis with hepatocellular carcinoma (170 cases), and hepatocellular carcinoma without cirrhosis (62 cases). The specimens were fixed in formalin, or sometimes in Zenker's formol and embedded in paraffin for histopathologic study. Sections were cut at 5 micron and stained with hematoxylin-eosin, resorcin fuchsin method for elastic fibers, Mallory's method for collagen fibers, silver impregnation method for reticulum fibers, and orcein method for hepatitis B surface antigen (Senba, 1982). The criteria of Leevy *et al.* (1979) and Gibson and Sobin (1978) were applied in assigning the diagnosis of cirrhosis an hepatocellular carcinoma to the tissue.

Statistical calculation was performed using the BMDP (Dixon *et al.*, 1981) on the IBM 4341 system in the Data Center of A-bomb Disaster in Nagasaki University. The statistical method for this study is the Pearson chi-square test for association in contingency table.

## Results

Table 1 shows the hepatitis B surface antigen positive results obtained by orcein staining. All of the 17 cases of acute hepatitis were not hepatitis B surface antigen positive. On the other hand, of the 26 chronic hepatitis cases in 1964–1973 and of the 14 in 1974–1983, 4 (15%) and 2 (14%) were hepatitis B surface antigen positive, respectively. As for the cirrhosis cases, 33 (33%) cases were hepatitis B surface antigen positive out of 100 in 1964–1973 and 8 (24%) out of 33 in 1974–1983. As for the cirrhosis with hepatocellular carcinoma cases, 77 (71%) cases were hepatitis B surface antigen positive out of 108 in 1964–1973 and 36 (58%) out of 62 in 1974–1983. Of the 21 and 41 hepatocellular carcinoma cases in 1964–1973 and 1974–1983, 5 (24%) and 11 (27%) were hepatitis B surface antigen positive, respectively.

Results of the statistical analysis on hepatitis B surface antigen, cirrhosis, and cirrhosis with hepatocellular carcinoma are as follows: The Pearson chi-square test for the frequencies of hepatitis B surface antigen positive in cirrhosis between the 2 periods showed Pearson $\chi^2=0.345$; and the Pearson chi-square test for the frequencies of hepatitis B surface antigen positive in cirrhosis with hepatocellular carcinoma between the 2 periods showed Pearson $\chi^2=0.079$, respectively. Thus, the difference of the hepatitis B surface antigen positive rates in cirrhosis

Table 1 Proportions of hepatitis B surface antigen carriers in hepatitis cases, cirrhosis cases, and hepatocellular carcinoma cases

| | 1964–1973 | 1974–1983 | Total |
|---|---|---|---|
| Acute hepatitis | 0/10 (0%) | 0/7 (0%) | 0/17 (0%) |
| Chronic hepatitis | 4/26 (15%) | 2/14 (14%) | 6/40 (15%) |
| Cirrhosis | 33/100 (33%) | 8/33 (24%) | 41/133 (31%) |
| Cirrhosis with HCC* | 77/108 (71%) | 36/62 (58%) | 113/170 (66%) |
| HCC* | 5/21 (24%) | 11/41 (27%) | 16/62 (26%) |
| Grand total | 119/265 (45%) | 57/157 (36%) | 176/422 (42%) |

* HCC: Hepatocellular carcinoma

with hepatocellular carcinoma between the 2 periods was marginally significant, but not significant in cirrhosis.

## Discussion

The landmark discovery of the Australia antigen (hepatitis B surface antigen) by Blumberg *et al.* (1965) and the linking of this antigen to viral hepatitis (Blumberg *et al.*, 1967) provided the cornerstone for the rapid acceleration on our understanding of viral hepatitis in general, and posttransfusion hepatitis in particular. The hepatitis B surface antigen positive cases were increased by such antigen detection, but false-positive reaction occurred frequently (Hollinger *et al.*, 1973). Further specific methods were developed for recognition of other hepatits B virus antigens (hepatitis B core antigen and hepatitis B e antigen). The introduction of sensitive assays for hepatitis B surface antigen has resulted in marked reduction in the incidence of posttransfusion hepatitis (Alter *et al.*, 1975). Therefore, in this study, proportions of hepatitis B surface antigen carrier in cirrhosis and cirrhosis with hepatocellular carcinorma in the recent decade 1974–1983 were found to decrease compared to the last decade 1964–1973. The decrease in the hepatitis B surface antigen positive rate from 45% to 36% suggests that infection of type B virus caused by posttransfusion can be prevented in the latter decade. However, the hepatitis B surface antigen positive rates in chronic hepatitis showed minor changes during 1964–1983. A possible explanation may be that high frequency of hepatitis B virus transmission from the carrier mother to her infant has occurred.

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# B型肝炎表層抗原の慢性肝炎，肝硬変および肝硬変に伴った肝細胞癌組織中の陽性率の減少傾向

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1964－1973年と1974－1983年の各年代におけるB型肝炎表層抗原の陽性率を急性肝炎，慢性肝炎，肝硬変，肝硬変に伴った肝細胞癌および肝細胞癌の組織を長崎大学病院の剖検肝臓422例を使用して検索した。B型肝炎表層抗原は急性肝炎の17例の全て陰性であり，慢性肝炎および肝細胞癌の例では1964－1983年の差は小さかった。また，B型肝炎表層抗原は肝硬変および肝硬変に伴った肝細胞癌の例で減少していた。しかしながら，2年代におけるB型肝炎表層抗原の減少は肝硬変に伴った肝細胞癌の例では有意であったが，肝硬変では有意ではなかった。このことは最近の10年間ではB型肝炎ウイルスによる輸血後肝炎がなくなったためと思われた。

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# MATURATION OF CHIKUNGUNYA VIRUS IN SALIVARY GLANDS OF INDONESIA STRAINS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* MOSQUITOES: AN ELECTRON MICROSCOPIC STUDY

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**Abstract:** Electron micrsocopic studies on the salivary glands of *Aedes aegypti* and *Aedes albopicuts* mosquitoes infected with chikungunya virus showed vacuolization in the cells of this organs and numerous chikungunya virions as crystalloid structures in intracytoplasmic area, revealing the spherical particle of 55–69 nm in diameter and the electron-dense internal structure of 41–48 nm in diameter. Virions were also found enormously in this apical cavities. No findings of virus budding from the cell surface membrane of chikungunya infected mosquito's salivary glands were obtained.

## INTRODUCTION

Chikungunya virus, an alphavirus genus of Togaviridae, is widely distributed in Southeast Asia and Africa. *Aedes aegypti* and *Aedes albopictus* are important vectors of this virus. In the transmission of the virus, the salivary glands of these mosquitoes play especially an important role in producing and maintaining a high titer of viruses throughout their life (Takahashi and Suzuki, 1979). Experimental studies showed that there were wide variations in susceptibility among different species of *Aedes* mosquitoes and different strains of the same species to transmit chikungunya virus (Mangiafico, 1971; Tesh *et al.*, 1976; McIntosh and Jupp, 1970; Yamanishi *et al.*, 1983). For the elucidation of the virus susceptibility, the growth of chikungunya virus in salivary glands of *Aedes aegypti* and *Aedes albopicuts* were electron-microscopically studied.

## MATERIALS AND METHODS

### *Mosquitoes*

*Aedes aegypti* (Surabaya strain) from mosquitoes collected at an urban area of Surabaya, and

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*Aedes albopictus* (Malang strain) from mosquitoes collected at a rural area of Malang, Indonesia, colonized in the first author S.S.'s laboratory since 1983, were used throughout this study. Eggs of these colonies were transferred to Japan in 1986 and recolonized. Mosquitoes were reared in an insectary maintained at 27°C, with 70–80% relative humidity and a 16 : 8 hr (L : D) photoperiod (Konishi and Yamanishi, 1986).

*Virus*

Chikungunya virus, African strain passaged in the BHK–21 monolayer cultures (Konishi and Hotta, 1979), was used for infection of the mosquitoes.

*Infection of mosquitoes*

Eight- to 10-day old mosquitoes were infected by feeding on blood-virus-mixture at a titer about $10^{7.9}$ focus forming units (FFU) per m*l* (Soekiman *et al.*, 1986b). Fully-engorged females were transferred to another cage and maintained with 0.7% sucrose. Mosquitoes on the 9th- and 12th-day postinfection were used for the electron microscopic observation.

*Electron microscopy*

For the transmission electron microscopic observation, each of the mosquitoes of 9th- and 12th-day postinfection were anesthetized by chilling. The salivary glands attached to the head were dissected in saline solution under a dissecting microscope. After washed with PBS, the specimens were fixed in 2% glutaraldehyde for 1–12 hrs at 4°C and postfixed in 1% osmium tetroxide for 2 hr, both in Millonig's phosphate buffered saline. After dehydration in a graded series of ethanol followed by toluene treatment, specimens were embedded in Epon 812 in BEEM capsules (Nissin EM Co.) for serial cross sectioning. For easy observation of the dissected mosquito's salivary glands, embedding was performed as previously described (Soekiman *et al.*, 1986b). After thin sectioning were done with the Porter-Blum MT–2B ultramicrotome (Sorval), the sections were stained with uranyl magnesium acetate and lead citrate and observed under a HS–9 electron microscope.

## Results

The proximal portion in the lateral lobe of salivary glands of *Aedes aegypti* showed a number of enveloped chikungunya viruses accumulated in the apical cavity (Figure 1A). Microvilli also protruded into the apical cavities (Figure 1B). At a magnification of 48,000 the diameter of virions were between 55–69 nm with electron-dense internal structure of 41–48 nm. Especially close to virion accumulated area (crystalloid structure), a large number of mitochondria were seen clearly (Figure 2). Observation underneath the surface membrane of the lateral lobe cell in the salivary glands of *Aedes aegypti* (Figure 3A) and *Aedes albopictus* (Figure 3B) showed enveloped chikungunya viruses clearly. No finding of the virus budding from the cell surface membrane in these regions was obtained (Figures 3A, 3B). Precursor particles of chikungunya virus (Higashi *et al.*, 1967; Matsumura *et al.*, 1972) were not clearly observed in these salivary glands.

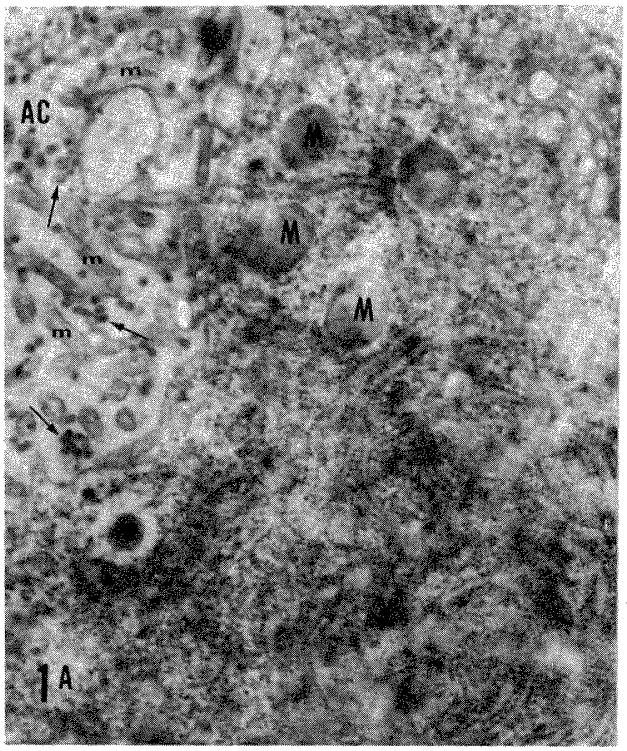


Figure 1A Thin section of proximal portion in the lateral lobe of salivary glands of *Aedes aegypti* infected with chikungunya virus by oral feeding showed a numerous number of virions in the apical cavity (Magnification: ×24,000; AC=apical cavity, M=mitochondria, m=microvilli. Arrows show virions).

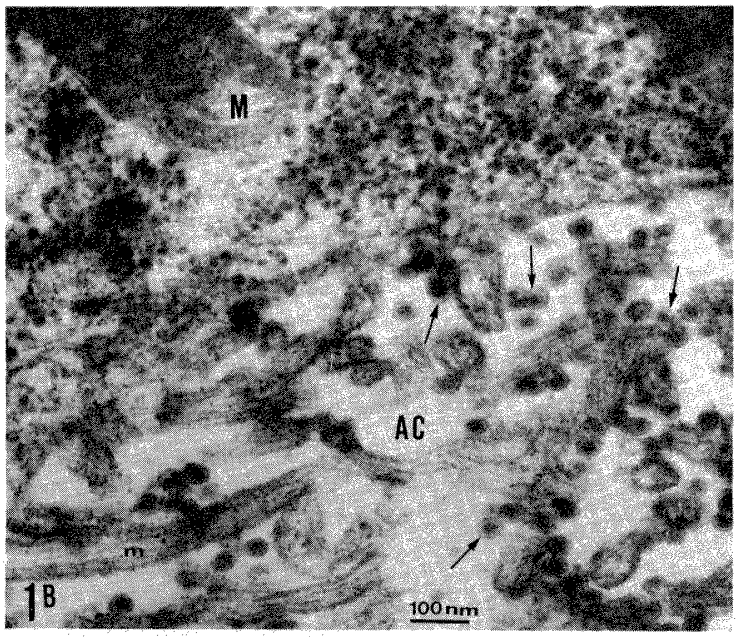


Figure 1B At a magnification of 48,000 the diameter of enveloped virions was between 55–69 nm, with electron-dense internal structure of 41–48 nm. Arrows show virions (AC= apical cavity, M=mitochondoria, m=microvilli).

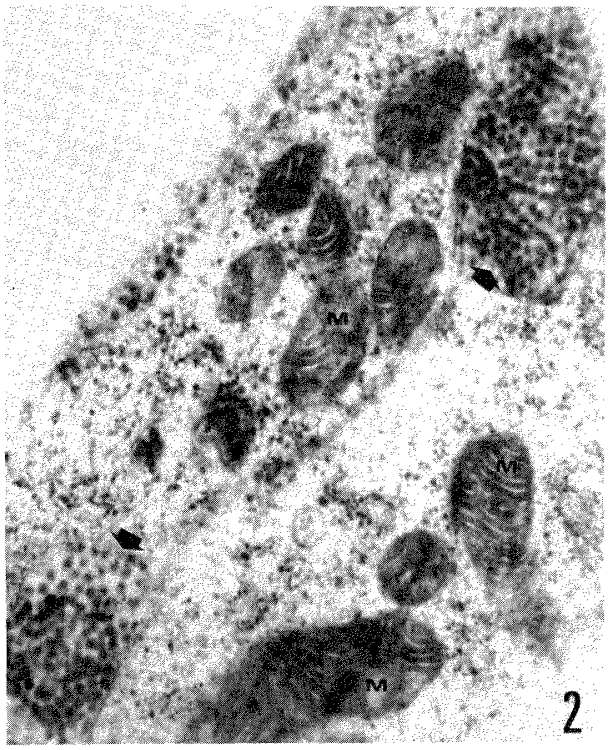


Figure 2 Close to the virion's crystalloid structure, a numerous number of mitochondria were clearly seen (*Aedes aegypti* salivary glands infected with chikungunya virus. Magnification: ×21,000; M=mitochondria. Arrows show virion's crystalloid structure).

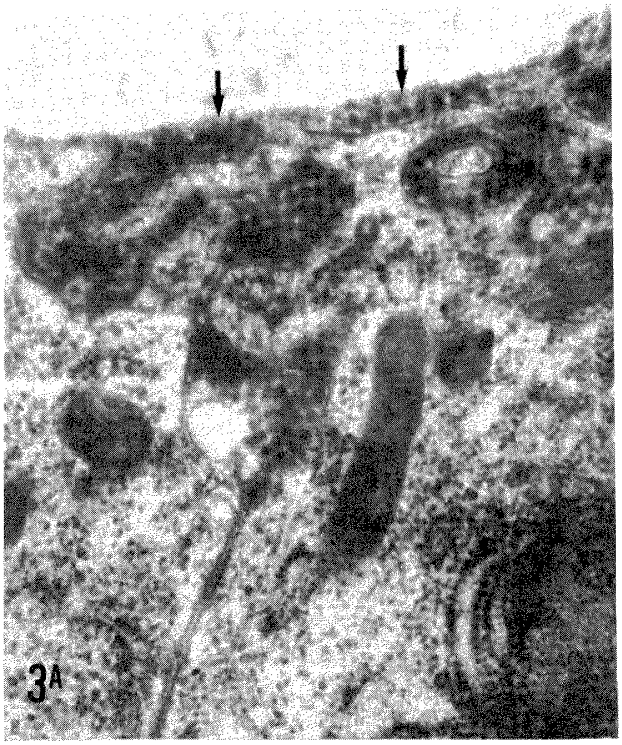


Figure 3A Mature enveloped chikungunya viruses lined along underneath the cell surface membrane (arrows), without any budding process (*Aedes aegypti* salivary glands; Magnification: ×28,000).

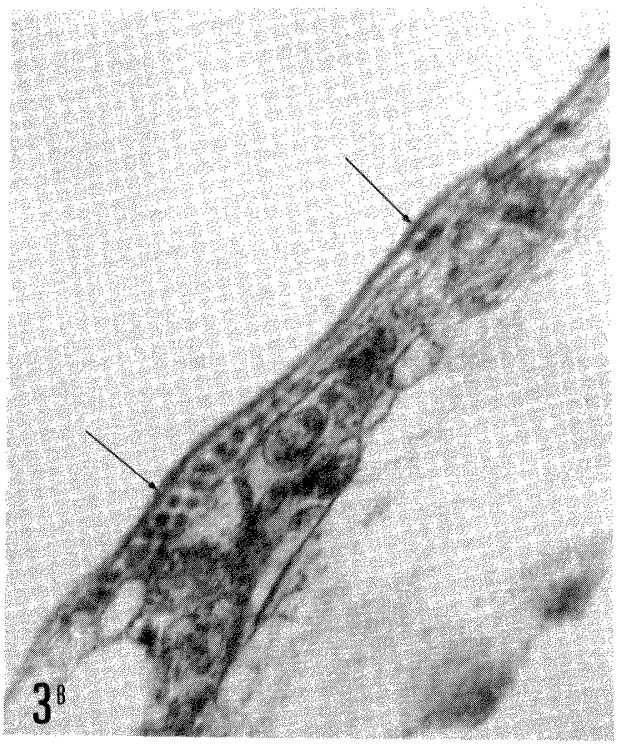


Figure 3B Salivary glands of *Aedes albopictus* infected with chikungunya virus by oral feeding showed mature virions underneath the cell surface membrane (arrows). No findings of virus budding from this salivary glands were also seen. Magnification: ×36,000.

## Discussion

In *Aedes aegypti* and *Aedes albopictus* infected with chikungunya virus by oral feeding, the titers of virus reached the peak on day 9 and 12, respectively (Soekiman *et al.*, 1986a). According to these results, electron microscopic observations were carried out in the mosquitoes of 9th-day postinfection in *Aedes aegypti* and 12th-day postinfection in *Aedes albopictus*.

Under the electron microscopic observation, the infected salivary glands showed increasing numbers of vacuoles. This vacuolization is a characteristic sign in mosquitoe's salivary glands infected with arbovirus by oral feeding, such as Japanese encephalitis virus in *Culex tritaeniorhynchus* (Takahashi and Suzuki, 1979) and dengue type-2 virus in *Aedes albopictus* mosquitoes (Sriurairatna and Bhamarapravati, 1977). This intensive vacuolization was also found in *Aedes albopictus* cell cultures infected with dengue viruses (Ko *et al.*, 1979) and Vero cell cultures infected with chikungunya virus (Higashi *et al.*, 1967).

Several reports have described chikungunya virus morphogenesis in cultured cells. In mammalian cell cultures such as Vero and BHK 21 cells, infected with this virus, the cell surface membrane was the site of the maturation process of the chikungunya virus (Higashi *et al.*, 1967; Matsumura *et al.*, 1972). Budding process can be seen commonly in this site and precursor particle of viruses were found underneath the cell surface membrane and around the vacuoles in the cytoplasma of the cells infected with the virus (Matsumura *et al.*, 1972; Matsumura and Hotta, 1978). Similarly cell-surface budding process was also seen in Vero, J-111 and IMR cell

cultures infected with chikungunya or dengue type 1 viruses (Ohyama *et al.*, 1977; Matsumura *et al.*, 1977) but it was not observed in BHK-21 cell lines infected with dengue type 1 and type 2 viruses (Hotta and Matsumura, 1979). Our study did not show virus budding from the cell surface membrane of the salivary glands of *Aedes aegypti* (Surabaya strain) and *Aedes albopictus* (Malang strain) infected with chikungunya virus. The observation in salivary glands of *Aedes albopictus* (Oahu strain) infected with chikungunya virus also did not show the budding process (Soekiman *et al.*, 1986b). Additionally the crystalloid structures of the virion as seen in Vero and BHK-21 cell cultures infected with dengue type 1 and type 2 viruses (Matsumura *et al.*, 1971; Matsumura *et al.*, 1977) were observed in the salivary gland of the infected mosquitoes.

The present study also showed that a numerous number of enveloped chikungunya viruses can be seen well in the intracytoplasmic areas of the salivary glands, and also were densely concentrated extracellularly in the apical cavities. Many mitochondria close to this crystalloid structures of the virus indicate that the viral replication may need these intracellular organella as the energy supplier (Hayashi, 1983). These results so far obtained suggested that in *Aedes aegypti* (Surabaya strain) as well as *Aedes albopictus* (Malang strain) infected with chikungunya virus, the virus maturation sites of salivary glands were intracytoplasmic membranous structure such as cysternae of endoplasmic reticulum and vesicles as already shown in *Aedes albopictus* (Oahu strain) salivary glands infected with chikungunya virus (Soekiman *et al.*, 1986b), Vero cells infected with dengue type 1 and type 2 viruses (Matsumura *et al.*, 1971) and *Culex tritaeniorhynchus* salivary glands infected with Japanese encephalitis virus (Takahashi and Suzuki, 1979). After maturation of the virus in the cytoplasm of the salivary glands, the virions might be released into the apical cavity by the normal secretion mechanism of saliva (Matsumura *et al.*, 1971; Matsumura and Yamashita, 1978). Electron-microscopically, there were no critical differences in the above-mentioned findings of the saliyary glands infected with chikungunya virus between *Aedes aegypti* (Surabaya strain) and *Aedes albopictus* (Malang and Oahu strains). It has not been clarified yet what does the difference of the maturation site of chikungunya virus between *in vivo* salivary glands of mosquitoes and mammalian cell cultures mean in relative to the ecology of this virus.

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# ネッタイシマカおよびヒトスジシマカ（インドネシア株）の唾液腺におけるチクングニアウイルス成熟の電子顕微鏡的研究

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チクングニアウイルスはトガウイルス科アルファウイルス属で，東南アジアをはじめアフリカなどに広く浸淫している。ネッタイシマカおよびヒトスジシマカは，本ウイルスの重要な媒介蚊である。ここでは，インドネシアで採取され，実験室で継代飼育されたネッタイシマカ（スラバヤ株），およびヒトスジシマカ（マラン株）を feeding 法で感染させ，両者の唾液腺における本ウイルスの成熟について電子顕微鏡観察を行った。感染後9日から12日の蚊では，ウイルス力価は最高値に達し，そのときの感染蚊の唾液腺細胞では空胞化が認められ，細胞質内には膜構造に囲まれたウイルス結晶構造が多数認められた。それらの粒子は，直径 55～69 nm で，電子密な直径 41～48 nm の内部構造を有していた。Virion は，唾液腺管腔内にも無数に遊離している像が観察された。両種の蚊には，特に異なった形態学的所見はなく，いずれも感染蚊唾液腺細胞の細胞表面膜からの出芽所見は認められなかった。

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**Short Communication**

# A COMPARATIVE STUDY ON GROWTH OF DENGUE TYPE 3 AND CHIKUNGUNYA VIRUSES IN INDONESIAN COLONIES OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* MOSQUITOES

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**Abstract:** The growth of dengue type 3 virus in 2 Indonesian colonies of *Aedes aegypti* (Surabaya strain) and *Aedes albopictus* (Malang strain) was studied in the oral infection system, compared with the growth of chikungunya virus in *Aedes aegypti* as a control. In all combinations of the virus and the host mosquito, maximum mean titers were as high as about $10^6$ to $10^8$ FFU per mosquito 8 to 12 days after infection. This result suggests that in addition to *Aedes aegypti*, *Aedes albopictus* is a possible important vector involved in dengue hemorrhagic fever epidemics in rural areas of Indonesia.

Dengue hemorrhagic fever (DHF) is a major health problem in Indonesia and other Southeast Asian countries (Hotta, 1978; Braude and Leelarasamee, 1981). Primarily DHF was one of the urban diseases, with most cases reported from larger cities in Java Island such as Surabaya and Jakarta, Indonesia. Since 1980, however, DHF epidemics have spread over smaller cities and rural areas in all provinces except Timor Timur. Virological surveillance revealed that dengue type 3 (DEN-3) virus, belonging to Flaviviridae, was the predominant virus isolated from the sera of DHF patients (Sunoto *et al.*, 1975; Gubler *et al.*, 1979b). *Aedes aegypti* and *Aedes albopictus* are common mosquito species in Indonesia. In urban areas, *Aedes aegypti* is a domestic mosquito usually found indoors, while *Aedes albopictus* is widely distributed outdoors in rural areas (Horsfall, 1955). A recent paper reported many breeding sites for *Aedes albopictus* outdoors, also in urban areas (Oda *et al.*, 1984). It is well known that there are wide variations in susceptibility to experimental infection with such mosquito-borne viruses among different species of mosquitoes and different strains of the same species (Gubler and Rosen, 1976; Tesh *et al.*, 1976; Gubler *et al.*, 1979a; Eshita, 1982; Yamanishi *et al.*, 1983). Our preliminary studies have indicated biological and ecological similarities between *Aedes aegypti* and *Aedes albopictus* collected in Indonesia (Soekiman *et al.*, 1984) and high susceptibility of both species to infection with chikungunya (CHIK) virus, an alphavirus also distributed in this nation (Soekiman *et al.*, 1986). In the present paper, the growth of DEN-3 virus in orally infected *Aedes aegypti* and *Aedes*

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*albopictus* was studied, in comparison with the CHIK virus growth in *Aedes aegypti.*

The mosquitoes employed were a colony of *Aedes aegypti* established in 1983 from specimens collected in Surabaya, an urban area of Indonesia, and a colony of *Aedes albopictus* originating from specimens collected in Malang rural area. Eggs of these colonies were taken to Japan in 1985 and recolonized. Mosquitoes were reared in an insectary maintained at 27°C, with 70–80% relative humidity and a 16 : 8 hr (L : D) photoperiod. Larvae were given a diet of mouse pellets and adult mosquitoes were provided with 0.7% sucrose. DEN-3 virus, H87 strain, was provided from the Department of Microbiology of our University at the 30th suckling mouse brain passage. After passed twice more in this laboratory, the seed virus was obtained from 10% homogenate of infected brain specimens in phosphate-buffered saline (PBS). CHIK virus, African strain (Konishi and Hotta, 1979), was also used in which the seed virus was the culture fluid obtained from infected BHK-21 monolayer cultures.

Feeding experiments and subsequent preparation of mosquito samples were performed as previously described (Konishi and Yamanishi, 1986). Four to 5 day-old adult female mosquitoes were allowed to feed on a mixture of the seed virus and defibrinated sheep blood with an infective titer of $10^{7.7}$ FFU (focus forming unit) per m*l* in DEN-3 virus and $10^{8.2}$ FFU per m*l* in CHIK virus. At 2-day intervals, 5 to 12 mosquitoes of each species were homogenized in 2.0 m*l* of culture medium, centrifuged at $700 \times g$ for 5 min, and then filtered through a 0.45 $\mu$m membrane to remove contaminating bacteria. The filtrate was assayed for viral infectivity by the indirect fluorescent antibody technique essentially based on the original focus-counting method by Igarashi and Mantani (1974). The first antibody was the DEN-3 monoclonal antibody obtained from culture fluid of the 5D4 hybridoma cell line (Henchal *et al.*, 1982) that was provided from the Department of Microbiology of our University, and the mouse anti-CHIK virus

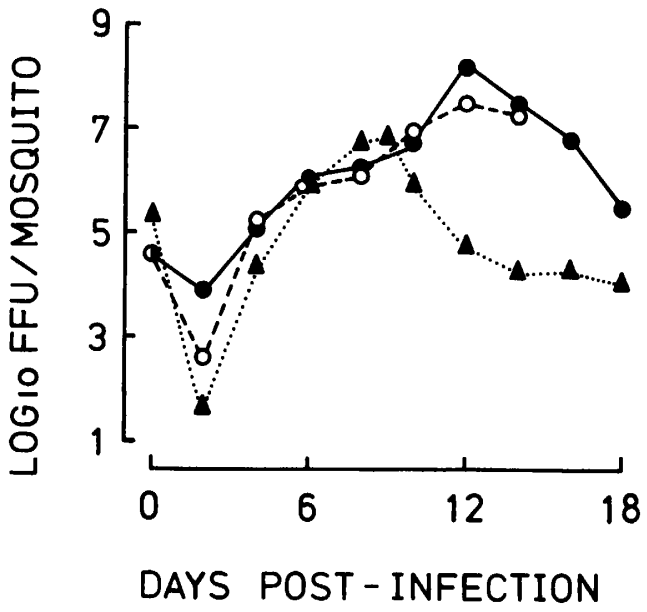


Figure 1 Growth of DEN-3 virus in Indonesia colonies of *Aedes aegypti* (●) and *Aedes albopictus* (○) after feeding on virus at $10^{7.7}$ FFU per m*l*, compared with growth of CHIK virus in *Aedes aegypti* (▲) after feeding on virus at $10^{8.2}$ FFU per m*l*. Mean virus titer obtained from 5–12 mosquitoes per pool was represented as FFU per mosquito.

antiserum obtained as described elsewhere (Konishi and Takahashi, 1985). The titer was expressed as FFU per mosquito.

The results are shown in Figure 1, where growth curves were obtained until 14 or 18 days after infection. The virus titer in *Aedes aegypti* infected with DEN-3 virus was $10^{4.6}$ FFU on day 0 (the datum immediately after feeding) and $10^{3.9}$ FFU on day 2, while in *Aedes albopictus* infected with DEN-3 virus the titer decreased from $10^{4.6}$ FFU on day 0 to $10^{2.6}$ FFU on day 2. The initial drop was remarkable in CHIK virus-infected *Aedes aegypti* in which titers on days 0 and 2 were $10^{5.4}$ FFU and $10^{1.7}$ FFU, respectively. The titer of DEN-3 virus reached a maximum of $10^{8.2}$ FFU in *Aedes aegypti* and $10^{7.5}$ FFU in *Aedes albopictus* both on day 12, in contrast to CHIK virus infection where the peak titer was $10^{6.9}$ FFU on day 9.

The initial drop observed in this study is consistent with other reports (Gubler *et al.*, 1979a; Yamanishi *et al.*, 1983). On the 2nd day of oral infection, no virus was recovered from hemolymph in CHIK virus-infected *Aedes aegypti* (Lanzen *et al.*, 1970), and DEN-2 virus antigen was limited to the posterior midgut cells of *Aedes albopictus* (Kuberski, 1979), suggesting the presence of "gut barrier" in the mosquito midgut. Subsequent virus growth indicates that these species were highly susceptible to experimental infection with DEN-3 and CHIK viruses. High DEN-3 virus titers observed in *Aedes aegypti* and *Aedes albopictus* were similar to those reported by Gubler and Rosen (1976) and Gubler *et al.* (1979a) with several geographic strains. Although susceptibility of *Aedes aegypti* to oral infection with CHIK virus was relatively low in general (Yamanishi and Konishi, data unpublished), the maximum titer obtained in this study was comparable to those of DEN-3 virus in *Aedes aegypti* and *Aedes albopictus*. The present data strongly support the opinion that *Aedes aegypti* is very important in the epidemiology of DHF as a potential vector of DEN-3 virus (Gubler *et al.*, 1979b; Tan *et al.*, 1981). However, it is also suggested from the population distribution of *Aedes* mosquitoes in Indonesia that *Aedes albopictus* is another important vector in DHF epidemics spreading over rural areas. Field studies including virus isolation from this species and its feeding pattern should be performed to verify the epidemiological importance of *Aedes albopictus*. Further studies to confirm the ability to transmit the virus are now in progress including the electron microscopic observation of the virus maturation process in the salivary gland of *Aedes aegypti* and *Aedes albopictus*.

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## 短　報

# インドネシア由来のネッタイシマカおよびヒトスジシマカにおけるデング3型ウイルスとチクングニアウイルス増殖の比較

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スラバヤ系ネッタイシマカにおけるチクングニアウイルス増殖を対照として，同蚊およびマラン系ヒトスジシマカにおけるデング3型ウイルスの増殖を経口感染により調べた。蚊の平均保有ウイルス量の最高値はいずれの組合せにおいても高く，感染後8日から12日目に約 $10^6$ から $10^8$ FFU を示した。この結果からインドネシアの農村部におけるデング出血熱の流行に関与する媒介蚊として，ネッタイシマカの他にヒトスジシマカも重要であることが示唆された。

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