日本熱帯医学会雑誌

第24巻 第1号 平成8年3月

内　　容

（裏面に続く）

日熱医会誌

日本熱帯医学会

Jpn. J. Trop. Med. Hyg., Vol. 24, No. 1, 1996, pp. 1-4

LOCALIZATION OF *TOXOPLASMA GONDII* ANTIGENS IN DENSE GRANULES AND RHOPTRIES DETECTED BY SERA FROM CONGENITAL TOXOPLASMOSIS PATIENTS

TATSUYA TEGOSHI[1], SHINJI MATSUDA[1], TAKASHI HAYANO[2], YOSHITSUGU MATSUMOTO[1], MINORU YAMADA[1], TSUNEZO SHIOTA[1], AKIO KURODA[1], AND NAOKI ARIZONO[1]

Received December 14, 1995/Accepted January 18, 1996

Abstract: Subcellular antigen localization in *Toxoplasma gondii* was studied by immunoelectron microscopy using sera obtained from congenital toxoplasmosis neonates or the mother. IgG and IgM antibodies bound specifically to the dense granules and rhoptries, but little bound to the cytoplasm of the organism or its tegmentum. The results indicate the importance of dense granules and rhoptries as a storage site for the antigens, which would be released and stimulate antibody production in human toxoplasmosis.

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite, which invades a wide variety of hosts and host cell types. Although many people infected with this organism are asymptomatic, infection during the pregnancy causes trasplacental transmission to the fetus and induces congenital toxoplasmosis. Various kinds of antigenic molecules have been isolated from *T. gondii*: tachyzoite membrane surface antigens (Johnson *et al.*, 1983), cytoplasmic antigens (Sharma *et al.*, 1984; Schwartzman, 1986; Sadak *et al.*, 1988), and excreted-secreted or circulating antigens (Van Knapen and Panggabean, 1977; Hughes and Van Knapen, 1982; Darcy *et al.*, 1988; Decoster *et al.*, 1988; Cesbron-Delauw *et al.*, 1989; Charif *et al.*, 1990). Antibodies raised in mice against 21-, 27-, and 28.5-kDa antigens were found to recognize dense granules as well as host cell-modified phagosomes (Charif *et al.*, 1990; Matsuura *et al.*, 1992). These results suggest that dense granules are an important storage site for antigens which may induce antibody responses at least in experimental animals. In human toxoplasmosis, however, the major target antigens and their subcellular localization in the organism have not been fully elucidated.

In the present study, we report the localization of antigenic molocules by immunoelectron microscopy that are recognized by sera obtained from congenital toxoplasmosis neonates and a mother.

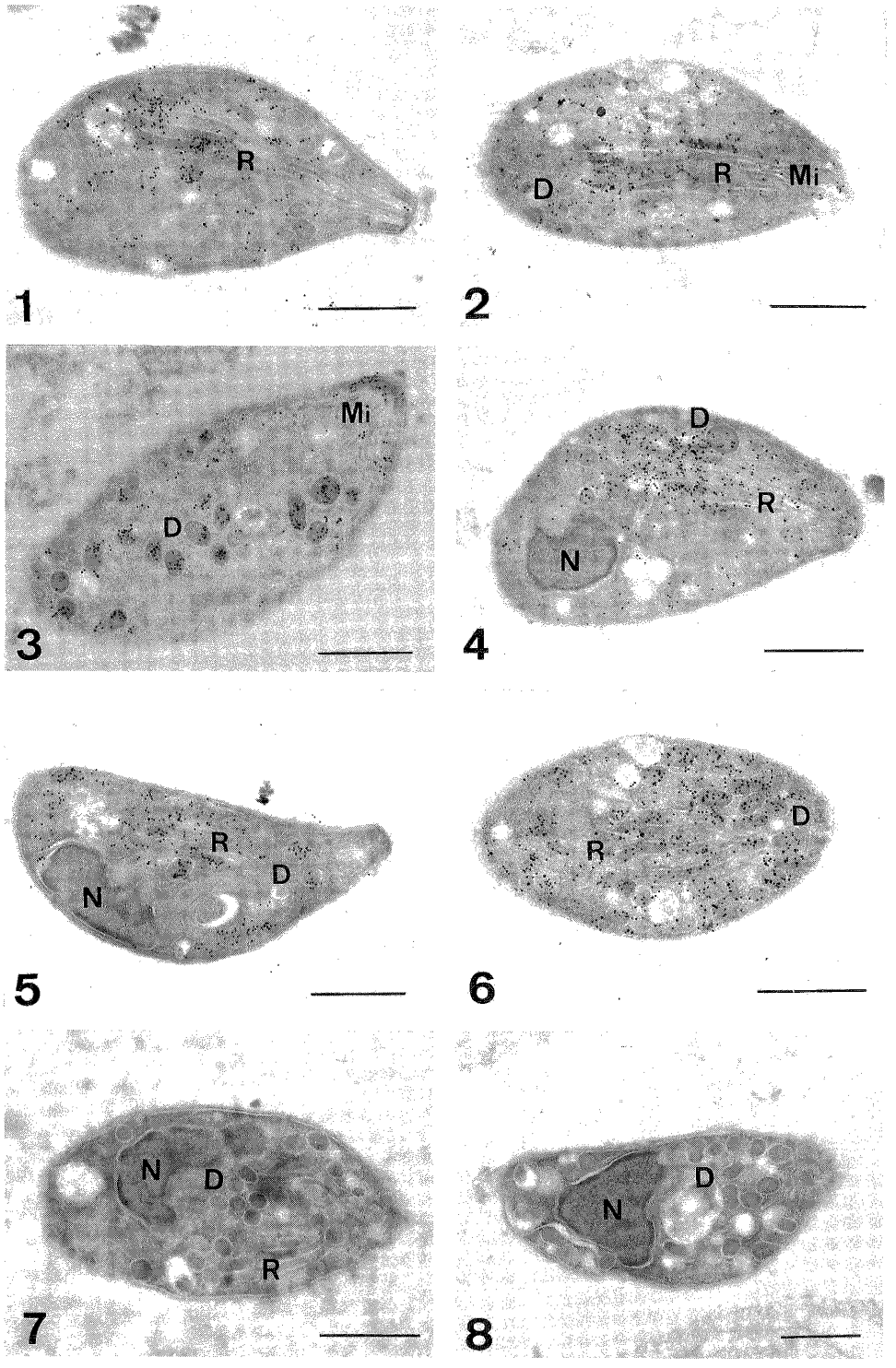
MATERIALS AND METHODS

Sera were obtained from two newborns with congenital toxoplasmosis and the mother who gave birth to one of them. Both babies showed hydrocephalus, retinochoroiditis, periventricular calcification, and some other neurological symptoms, while the mother was asymptomatic. Dye-test titers were 1:4096 in the babies and the mother. Normal sera were obtained from young adult volunteers whose dye-test titers were less than 1:4.

Two strains of *T. gondii*, RH and SK, were used in this study, the latter of which was isolated from the cerebrospinal fluid of one of the babies. Both strains were maintained by serial intraperitoneal inoculation in ICR mice.

For immunoelectron microscopy, tachyzoites obtained from mice were washed with 0.1M phosphate buffered saline (PBS) and fixed for 30 min at room temperature with a solution containing 1% paraformaldehyde and 0.1% glutaraldehyde in 0.1M phosphate buffer (PB), pH 7.4. Samples were washed with PB,

[1]Department of Medical Zoology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kyoto 602 Japan
[2]Division of Pediatrics, Children's Research Hospital, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kyoto 602 Japan



Figures 1-8 Subcellular localization of *Toxoplasma gondii* antigens recognized by sera from congenital toxoplasmosis neonates and a mother. Sections of *T. gondii* were incubated with sera from toxoplasmosis babies (Fig. 1-4), serum from a mother of toxoplasmosis baby (Figs. 5, 6), serum from a healthy volunteer (Fig. 7), or only with PBS (Fig. 8). The specimens were then incubated with rabbit anti-human IgG (Figs. 1, 2, 3, 5, 6, 7, 8) or with anti-human IgM (Fig. 4), and finally reacted with colloidal gold-conjugated goat anti-rabbit IgG. In Figs. 1-6, gold particles are localized on the rhoptries (R), dense granules (D) and sparsely on micronemes (Mi). N: nucleus Bar=1 μm

dehydrated in an ethanol series, and embedded in LR White resin (London Resin Co., Surrey, U.K.). After polymerization at 37°C for 5 days, ultrathin sections were cut, mounted on nickel grids, and etched for 1 h at 37°C with sodium metaperiodate saturated aqueous solution. The grids were rinsed with distilled water and blocked with 5% (w/v) non-fat dried milk in PBS, pH 7.4, containing 0.01% (v/v) Tween-20 for 30 min at room temperature. Sections were incubated for 2 h at room temperature with patients' sera (diluted 1:125) as the first antibody. As a negative control, normal sera from healthy adults were applied. Additional negative control sections were incubated only with PBS. The grids were rinsed with PBS containing 0.01% Tween-20 (PBS-Tween), and then incubated with rabbit anti-human IgG, IgM or total Igs (diluted 1:100; Cappel, Westchester, PA) for 1 h at room temperature. Sections were then washed and incubated with goat anti-rabbit IgG conjugated with colloidal-gold particles (15 nm; Janssen Life Science Products, Beerse, Belgium) for 1 h at room temperature. The grids were washed with PBS-Tween, stained in 2% uranyl acetate, carbon-coated, and then observed under a JEOL 100S electron microscope.

Results and Discussion

Using the babies' sera, immunogold labeling was observed mainly over the matrix of dense granules (Fig. 2, 3, 4) and rhoptries (Fig. 1, 2, 4), and sparsely on micronemes (Fig. 2, 3). Serum from the mother showed the similar gold labeling to these organelles (Fig. 5, 6). There were no significant differences for IgG (Fig. 1, 2, 3, 5, 6) and IgM (Fig. 4) in the labeling pattern, or between RH and SK strains. Sections incubated with normal sera (Fig. 7) or control sections without primary antibody (Fig. 8) were unlabeled.

The present immunoelectron microscopic study showed that antibodies from both the neonates with congenital toxoplasmosis and the asymptomatic mother mainly recognized dense granules and rhoptries with little labeling in cytoplasm and tegmentum of the organism.

It has been reported that rhoptries contain antigens of 55- and 60-kDa, that are suggested to be secreted (Sadak *et al.*, 1988; Schwartzman and Krug, 1989). Dense granules also seem to have antigens that would be released after host cell invasion; the 21-, 27-, and 28.5-kDa antigens were identified in dense granules as well as in the network of microvilli present within the parasitophorous vacuoles, (Charif *et al.*, 1990). Furthermore, secretory antigens were observed in the serum within one day after infection (Van Knapen and Panggabean, 1977), and were recognized by human sera from toxoplasmosis patients (Decoster *et al.*, 1988). At least some of the antibodies raised against secretory antigens seem to be protective, since these antigens reportedly induced both antibody-mediated and cell-mediated protective immunity against *T. gondii* infection in nude athymic rats (Darcy *et al.*, 1988).

The present results indicated that rhoptries and dense granules are major sites for antigens which might be secrete when *T. gondii* invades into host cells and induce antibody response in human toxoplasmosis.

References

1. Cesbron-Delauw, M.F., Guy, B., Torpier, G., Pierce, R.J., Lenzen, G., Cesbron, J.Y., Charif, H., Lepage, P., Darcy, F., Lecocq, J.P. and Capron, A. (1989): Molecular characterization of a 23-kilodalton major antigen secreted by *Toxoplasma gondii*, Proc. Natl. Acad. Sci., U. S.A. 86, 7537-7541
2. Charif, H., Darcy, F., Torpier, G., Cesbron-Delauw, M.F. and Capron, A. (1990): *Toxoplasma gondii*: characterization and localization of antigens secreted from tachyzoites, Exp. Parasitol., 71, 114-124
3. Darcy, F., Deslee, D., Santoro, F., Charif, H., Auriault, C., Decoster, A., Duquesne, V. and Capron, A. (1988): Induction of a protective antibody-independent response against toxoplasmosis by in vitro excrete/secreted antigens from tachyzoites of *Toxoplasma gondii,* Parasite. Immun. 10, 553-567
4. Decoster, A., Darcy, F. and Capron, A. (1988): Recognition of *Toxoplasma gondii* excreted and secreted antigens by human sera from acquired and congenital toxoplasmosis: identification of markers of acute and chronic infection, Clin. Exp. Immunol., 73, 376-382
5. Hughes, H.P.A. and Van Knapen, F. (1982): Characterization of a secretory antigen from *Toxoplasma gondii* and its role in circulating antigen production, Int. J. Parasitol., 12, 433-437
6. Johnson, A.M., McDonald, P.J. and Neoh, S.H. (1983): Monoclonal antibodies to *Toxoplasma gondii* cell membrane surface antigens protect mice from toxoplasmosis, J. Protozool., 30, 351-356
7. Matsuura, T., Tegoshi, T., Furuta-Matsuura, M. and Sugane, K. (1992): Epitope-selected monospecific antibodies to recombinant antigens from *Toxoplasma gondii* reacted with dense granules of tachyzoites, J. Hisochem. Cytochem., 40, 1725-1730
8. Sadak, A., Taghy, Z., Fortier, B. and Dubremetz, J.F. (1988): Characterization of a family of rhoptry proteins of *Toxoplasma gondii,* Mol. Biochem. Parasitol., 29, 203-211
9. Schwartzman, J.D. (1986): Inhibition of a penetration-

enhancing factor of *Toxoplasma gondii* by monoclonal antibodies specific for rhoptries, Infect. Immun., 51, 760-764

10. Schwartzman, J.D. and Krug, E. (1989): *Toxoplasma gondii*: Characterization of monoclonal antibodies that recognized rhoptries, Exp. Parasitol., 68, 74-82
11. Sgarma, D.D., Araujo, F.G. and Remington, J.S. (1984): Toxoplasma antigen isolated by affinity chromatography with monoclonal antiboby protects mice against lethal infection with *Toxoplasma gondii*, J. Immunol., 133, 2818-2820
12. Van Knapen, F. and Panggabean, S.O. (1977): Detection of circulating antigen during acute infection with *Toxoplasma gondii* by enzyme-linked immunosorbent assay, J. Clin. Microbiol., 6, 545-547

Jpn. J. Trop. Med Hyg., Vol. 24, No. 1 1996, pp. 5-9

EFFECT OF DEOXYSPERGUALIN ON THE GROWTH OF *PLASMODIUM BERGHEI* IN MICE

YUTAKA MIDORIKAWA[1], AND QUAZI MANJURUL HAQUE[2]

Received December 14, 1995/Accepted February 20, 1996

Abstract: Effects of deoxyspergualine (DSG), an immunosuppressive agent and polyamine synthesis inhibitor, on the rodent malarial protozoa *Plasmodium berghei* development was studied *in vivo*. DSG at doses of 5mg/kg body weight (b.w.) was injected intraperitonealy into mice (Balb/c) that had been infected with *P. berghei* (ANKA strain). DSG injection showed to be effective in reducing the percentage of parasitemia and spleen weight and in prolonging the survival days of infected mice. These findings in this study suggest the possibility that DSG could be developed as a candidate for anti-malarial drugs.

Key words: Deoxyspergualin, Malaria, *Plasmodium berghei*

INTRODUCTION

Since the resistance of *Plasmodium falciparum* (Harinasuta *et al.*, 1962) to almost all type of anti-malarial drugs was shown to have appeared, the development of a new type of effective anti-malarial drug have been desired (Arnold *et al.*, 1990). We had tried to use polyamine inhibitors for the anti-malarial drugs in this study.

Polyamines play important roles in the proliferation of cellular DNA, RNA and protein synthesis as well as the cell synthesis. Poliamine inhibitors inhibited the growth of some bacteria species (Midorikawa *et al.*, 1991). Deoxyspergualin (DSG) (Iwasawa *et al.*, 1982), a potent immunosuppressive agent (Suzuki *et al.*, 1987), was proved to be an inhibitor of polyamine synthesis. We have demonstrated that DSG showed antimicrobial activity on some bacterial strains (Hibasami *et al.*, 1991). In the present study, we investigated the effects of DSG on proliferation of a rodent malarial parasite, *Plasmodium berghei*.

MATERIALS AND METHODS

Malarial strain

In vivo culture of *Plasmodium berghei* (*P. berghei*) ANKA strain kept in the liguid N_2 was used through out the present experiment (Matsuoka *et al.*, 1992).

Experimental animals

Erythrocytes that have been infected with *P. berghei*, (% parasitemias were 5%), were injected to mice (Balb/c) intraperitoneally (i.p.).

Chemicals

DSG was produced by Takara Shuzo Co. LTD., Kyoto Japan. Solution of DSG for injection was prepared in distilled water and was sterilized through 0.45 millipore membrane filter. Solutions of various concentrations of DSG and quinine HCl was prepared for injection as follows (Watt *et al.*, 1992).

Procedure of study

Thirty mice were divided into 5 groups of Group A to E (6 mice per each) as follows for the first experiment.

A: Control mice, infected with *P. berghei*.
B: Infected mice injected with 5.0 mg DSG/kg/day for 6 days.
C: Infected mice injected with 2.5mg DSG/kg/day for 6 days.
D: Infected mice injected with 2.5mg quinine/kg/day hydrochloride for 6 days.
E: Non-infected mice injected with 5mg DSG/kg/day for 6 days.

Four mice for Group A and six mice for other treatments (B to C) were used in each group. DSG and quinine HCl were administered once a day for 6 days to the mice. These treatments were started when 10 days

[1]Depatment of Medical Nutrition, Suzuka University of Medical Sciences and Technology
[2]Department of Public Health, Schoool of Medicine, Mie University

had passed and % parasitemia became 2 to 10% following the injection of mice (i.p.) with *P. berghei*. Percentage parasitemia and survival days after the first treatment were noted in this experiment.

In the second experiment, 18 mice infected with *P. berghei* were divided into 3 groups that had the treatment as follows.

A: DSG (2.5mg/kg/day) administrated daily for 12 days.

B: DSG (2.5mg/kg/day) administrated on every other day for 12 days.

C: Quinine HCl (25mg/kg/day) administrated daily for 12 days.

Method of injection of DSG and quinine HCl was similar to that in the first experiment. Percentage parasitemia of each mouse was noted since the first treatment of drug.

In the third experiment, 6 infected mice with *P. berghei* were used. In this case, the administration of DSG (2.5mg/kg) was started at the time when the infected erythrocytes appeared in the mouse and stopped when the infected malarial parasites had disappeared in the blood of each mouse. Then, when parasite appeared again, the administration of DSG was started again until % parasitemia decreased to 0%. Administration of DSG was started when the parasites appeared again.

Spleen weights

Six mice were infected with *P. berghei* on the same day. After the infection of parasite was confirmed, these mice were divided into 2 groups. In one group DSG treatment (2.5mg/kg, i.p.) was continued until all the mice became 0% parasitemia. Another group did not recieved any treatment. Then 5 days ofter the first treatment, spleen weight of all mice were measured. The spleen of 3 non-infected mice were also weighed as controls.

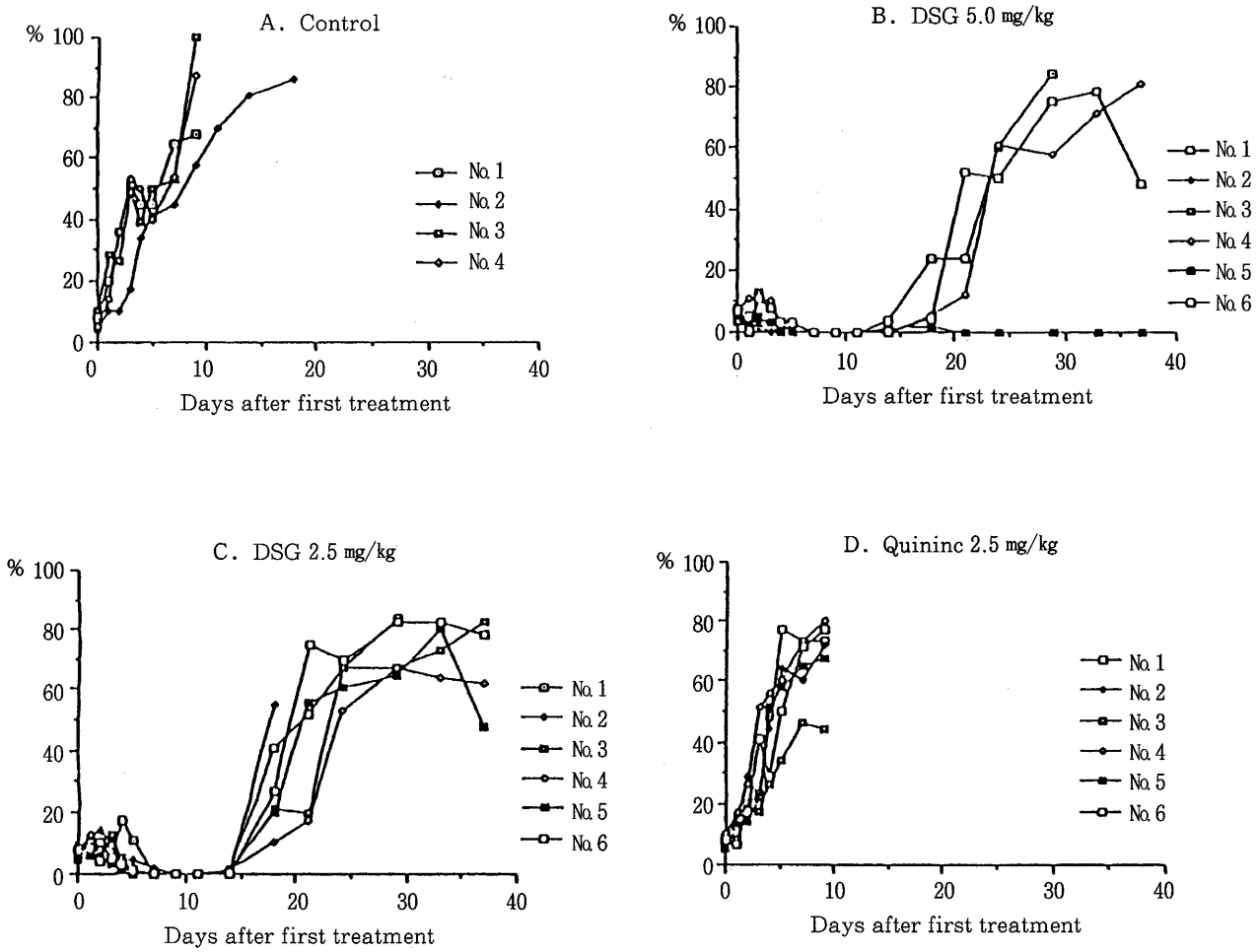


Figure 1 Effects of DSG and Quinine treatment on increasing the % parsitemia of *P. berghei* and survival days after the first treatment in first experiment.

RESULTS

Twenty-two out of 24 mice inoculated with *P. berghei* were positive for the parasite in smear of the erythrocyte. It was after 10 days from the inoculation of *P. berghei*. As the remaining 2 mice were not able to be infected well with *P. berghei* (too late to increase the parasitemia) they were excluded from group A. Then the number of mice in group A (control) had 4. Other 4 groups had 6 mice each. The first treatment DSG and quinine HCl had been done ten days after inoculation with *P. berghei*. Fig. 1 shows the change of % parasitemia and the lenght of survival time from the first treatment. Three out of four mice infected but given no drug treatment (group A) survived less than 10 days after the first treatment (20 days after infection). Only one mouse survived nearly 20 days after the first treatment (Fig. 1A). This means that all of mice without DSG that infected by *P. berghei* died in 30 days after infection of *P. berghei*. Administration of 2.5mg and 5.0mg DSG/kg/day for 6 days, both decreased the parasitemia to 0% by 5 days after the first treatment and markedly prolonged the survival time (Fig. 1B and 1C). Some mice died after the rapid decrease in % parasitemia due to DSG administration (Fig. 1B No. 3, 4 & Fig. 1C No. 1). Other mice survived nearly 40 days after the first treatment. The survival time of quinine-treated mice was less than 10 days, and this result was similar as that in non-treated of control (Fig. 1D).

Quinine HCl showed no effect on the infection of *P. berghei*. Non-infected mice and injected with of 5mg DSG/kg in Group E showed no major adverse effects such as diarrhea, decrease of body weight and the abnormal behavioral changes by DSG. All the mice belonging to group E survived for a long period after this experiment finished (data not shown).

Results of the second experiment were shown in Fig. 2. DSG treatment increased the survival time of infected mice of group A following the prolongation of the period of DSG administration that was from 6 days to 12 days (Compare Fig. 1C and Fig. 2A). The survival time of that was prolonged by longer treatment, in other

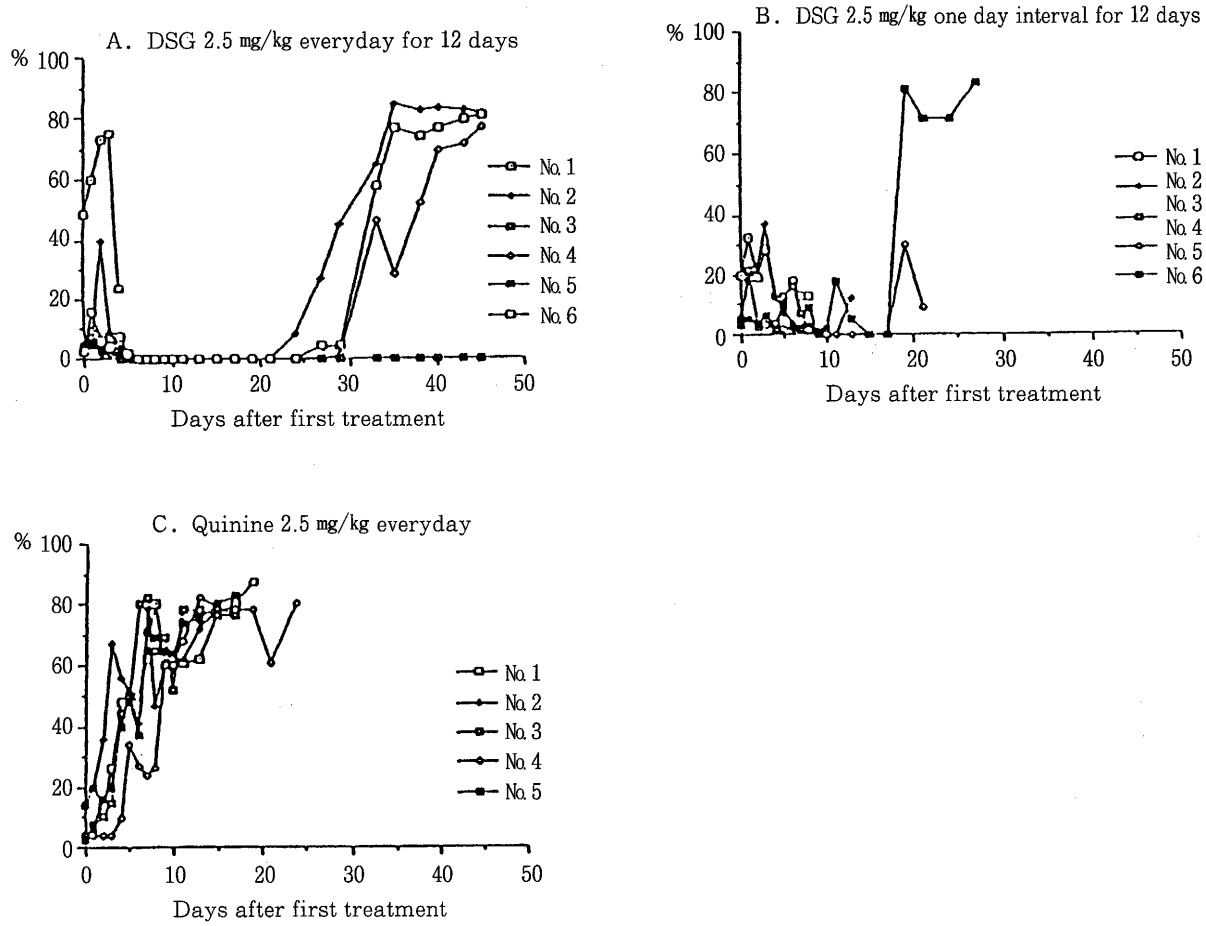


Figure 2 Effects of DSG and Quinine treatment on increasing the % parasitemia of *P. berghei* and survival days after the first treatment in second experiment.

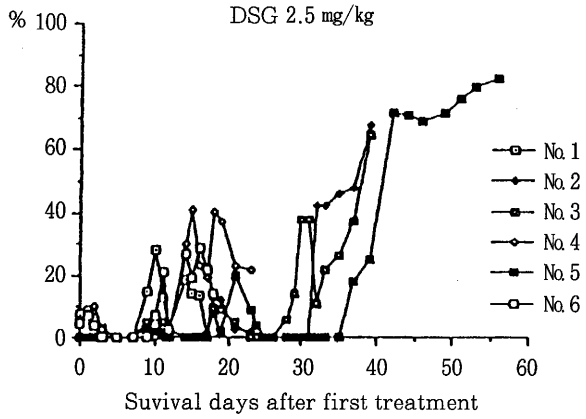


Figure 3 Effects of DSG treatment on increasing the % parasitemia of *P. berghei* and survival days from the first treatment in third experiment.

words, prolonged days (6 days) of DSG treatment. Though DSG treatment at one day interval for 12 days decreased the % parasitemia in group B, the survival time of mice did not increase. Because in this experiment, most mice died following the rapid decrease in % parasitemia due to the effect of DSG treatment (Fig. 2B). Dose of quinine HCl in group C was increased ten times more than that in Group E of the first experiment. However the result was not different from that without DSG treatment (Fig. 2C).

In the third experiment, DSG treatmeent was repeated when malarial parasite appeared again in the erythrocyte of each mouse. By this treatment, % parasitemia decreased following the DSG treatment and survival days were increased (Fig. 3).

Weights of spleens of the mice without DSG treatment were increased by the infection of *P. berghei*. DSG treatment reduced the weight of spleen of infected mice being at the level of that of non-infected mice when the % parasitemia decreased to 0 % by DSG administration (Tab. 1).

DISCUSSION

Malaria is endemic or sporadic throughout most of the tropics and subtropics. About one hundred million people are infected and 1% of them die annually. An incidence of the disease is increasing in the world. The development of resistant parasites against anti-malarial drugs is also increasing throughout the world (Storchler, 1989, WHO, 1986, Moran and Bernard, 1989). There are many problems due to currently used malarial drugs (Miller *et al.*, 1986). So, DSG was a candidate for new treatment in this research.

DSG treatment decreased the percentage parasitemia and prolonged the survival time that was shown in the first experiment. Prolonged DSG treatment in second experiment increased the survival time than that of first experiment. This fact means that these mice were prevented from relapse of malarial parasite for some days by prolonged DSG treatment.

Morphological change of shape of protozoa and property of stain by DSG treatment was not notable in this experiment.

We have demonstrated that DSG showed antimicrobial activity on some bacterial strains in vitro (Hibasami *et al.*, 1991). As this previous experiment of some bacterial strains, DSG treatment decreased parasitemia of *P. berghei* and resulted to increase the survival time of the mice. Inhibition of polyamine synthesis of DSG can explain the mechanism of decreasing % parasitemia of *P. berghei*. DSG treatment of 5mg per kg showed no major adverse effects. As far as this result is concerned, DSG itself did not show toxity to mice at this dose.

Survival time of DSG treated mouse was longer than that of quinine HCl treatment. It can be said that quinine HCl was not effective for ANKA strain of *P. berghei* in this experiment. It can say that DSG was more effective than quinine HCl in this study.

DSG treatment prevented mice from the spleen enlargement by *P. berghei* infection. This possibly resulted from the suppression of lymphoid hyperplasia by DSG.

Thus, decreasing % parasitemia, increasing the survival time and decreasing the spleen weight in infected mice were clearly evidenced as this conclusion. Effects of DSG on the asexual stage of *P. berghei* were

Table 1 Weight of spleen of mice with and without DSG treatment

Treatment	% Parasitemia	Weight of spleen ± SD
infected mice with DSG treatment	0	0.155 ± 0.057
infected mice without DSG treatment	10 ± 3.1	0.554 ± 0.370
not infected mice without DSG treatment	−	0.134 ± 0.025

Percent parasitemia 5 days after first treatment of DSG

shown in vivo in this study. So, further studies on the mechanism of anti-malarial effect of DSG will be needed for developing new type of anti-malarial drug.

ACKNOWLEDGMENTS

This study was supported by grant-in Aid (No. 04770332, 1992) for Scientific Research from Ministry of education Japan. We thank Prof. Hiroji Kanbara Institute of Tropical Medicine Nagasaki University for many suggestions.

REFERECES

1) Arnold, K., Hien, T.T., Chinh, N.T., Phu, N.H. and Mai, P.P., (1990): A randomized comparative stud of artemisinine (Qinghaosu) suppositories and oral quinine in acute falciparum malaria. Trans, R. Soc. Trop. Med. Hyg., 84, 499-502

2) Harinasuta, T., Migasena, S. and Boonnag, D. (1962): Chloraquine resistance in *Plasmodium falciparum* in Thailand. UNESCO First Regional Symposium on Scientific Knowledge of Tropical Parasites. Univ. Singapore, November 5-9, 1962, 148-153

3) Hibasami, H., Midorikawa, Y., Gasaluck, P., Yoshimura, H., Takaji, S., Nakashima, K. and Imai M, (1991). Bactericidal effect of 15-deoxysper-gualin on *Staphylococcus aureus*. Chemothera. 37, 202-205

4) Iwasawa, H., Kondo, S., Ikeda, D., Takeuchi, T., Umezawa, H., (1982): Synthesis of (-)-15-deoxyspergualin and (-)-spergualin-15-phosphate. J. Antibi. 34, 1619-1621

5) Matsuoka, H., Yamamoto, S., Chinzei, Y., Ando, K., Arakawa, R., Kamimura, Syafruddin, K., Kawamoto, F. and Ishii, A., (1992): Cyclical transmission of *Plasmodium berghei* (*coccidiida: Plasmodiidae*) by *Anopheles omorii* (*Diptera: Culicidae*). J. Med. Entom., 29, 343-345

6) Midorikawa, Y., Hibasami, H., Basaluck, P., Yoshimura, T., Nakashima, K. and Imai, M., (1991): Evaluation of the anti-microbial activity of methylglyoxal bis (guanyl-hydrazone analogues, the inhibitors for polyamine biosynthetic pathway. J. Appl. Bact., 70, 291-293

7) Miller, K.D., Lobel, H.O., Satriale, R.F., Kuritsky, J.N., Stern, R. and Campbell, C.C. (1986): Severe cutaneous reactions among American travelaers using pyrimethamine-sulfadoxine (Fansidar) for malaria prophylaxix. Am. J. Trop. Med. hyg., 35, 451-458

8) Moran, J.S. and Bernard, K W (1989). The spread of chloroquine-resistant malaria in Africa. Implications for travelars. JAMA., 262, 245-248

9) Storchler, D (1989): How much malaria is there world wide ? Parasite Today., 5, 39-40

10) Suzuki, S., Kanashiro, M. and Amemiya, H., (1987). Effect of a new immuno-suppressant 15-deoxyspergualin, on hetero-topic rat heart transplantation, in comparison with cyclosporine. Transplant. 44, 483-487

11) Watt, G., Loesuttivibool, L., Shanks, G. D., Boudreau, E. F., Brown, A. E., Pavanand, K., Webster, H.K. and Wechgritaya, S., (1992): Quinine with tetra-cycline for the treatment of drug-resistant falciparum malaria Thailand. Am. J. Trop. Med. Hyg., 47, 108-111

12) WHO, (1986): Severe and complicated malaria. Trans. Roy. Soc. Trop. Med. Hyg., 80, 1-50

Jpn. J. Trop. Med. Hyg., Vol. 24, No. 1, 1996, pp. 11-19

COMPUTER SIMULATION OF A MALARIA CONTROL TRIAL IN VANUATU USING A MATHEMATICAL MODEL WITH VARIABLE VECTORIAL CAPACITY

HIROFUMI ISHIKAWA[1], AKIRA KANEKO[2] and AKIRA ISHII[3]

Received December 13, 1995/Accepted February 24, 1996

Abstract: We have developed a mathematical model to estimate the degree of transmission of *Plasmodium falciparum* malaria, that is adjusted to an endemic region in Vanuatu, eastern Melanesia, incorporating the factor concerning the loss of immunity in human hosts. Applying this model, we have carried out simulations for more than fifty situations. Our model is based on Dietz *et al.* (DMT) model which contains epidemiological factors related to malaria transmission. By using field data from an epidemiological study in Vanuatu, we have determined epidemiological parameters and improved the model. Also our model can treat situations with variable vectorial capacity value. The simulations carried out show that it is important to decrease vectorial capacity by taking anti-malarial measures in order to reduce the prevalence of malaria. One or two operations of mass drug administration will reduce *Plasmodium falciparum* prevalence drastically, but intense reinfection will raise infection rate again within a few years. However, the resurgence of malaria will not occur for a long time if mass drug administration accompanied with possible reduction of vectorial capacity by vector control such as insecticide impregnated bed nets and others.

Key words: malaria, mass drug administration, mathematical model, *Plasmodium falciparum*, Vanuatu

INTRODUCTION

Vanuatu is a country that consists of more than eighty islands, and is located in eastern Melanesia, southern Pacific Ocean. *Plasmodium falciparum* and *Plasmodium vivax* are endemic in Vanuatu. We reasonably regard an island as a bioecologically isolated region, which affords a suitable situation for the epidemiological study based on a mathematical model.

To interrupt the transmission of malaria, mass drug administration is one of the valid and effective methods. A gametocidal drug eliminates the sexual stages of the parasite; a schizonticidal drug protects the individual infected with malaria against the asexual stages of the parasite. In the Vanuatu context (Kaneko *et al.*, 1994), they used both a gametocidal drug (primaquine) and schizonticidal drugs (chloroquine and Fansidar). Malaria transmission forms a cycle between a human and a mosquito. By biting, a mosquito ingests gametocytes in the human who has parasites in the blood. A subsequent bite of the vector can make a human infected through the intrusion of sporozoite. Consequently, the prevalence of malaria can be eradicated or reduced if, by physical, chemical and/or biological methods, we cut a part of the transmission cycle or lower the passing rate in any stages of the cycle.

In the present paper, we have developed a mathematical model for *Plasmodium falciparum* malaria transmission that is fit for Vanuatu epidemic region, based on an approach, DMT model, described by Dietz, Molineaux and Thomas who treated the case of the Africa Savannah (Dietz *et al.*, 1974). The Collett-Lye model (Collett and Lye, 1987) that developed from DMT model has also furnished us with much information. Our model is formed as a system of non-linear difference equations. Our model can also treat situations with variable vectorial capacity value. The parameters used in our model have been chosen on the basis of the epidemiological data in Vanuatu collected by the second author *et al.* (Kaneko *et al.*, 1994). For various situations——"How many times will mass drug administration be carried out? Will the distribution of permethrin

[1]Department of Environmental and Mathematical Sciences, Faculty of Environmental Science and Technology, Okayama University, Tsushimanaka, Okayama 700, Japan
[2]Department of International Affairs and Tropical Medicine, Tokyo Women's Medical College, Kawada, Shinjuku, Tokyo 162, Japan
[3]Department of Medical Zoology, Jichi Medical School, Yakushiji, Minamikawachi, Kawachigun, Tochigi, 329-04, Japan

impregnated bed nets be carried out?"——we have produced the simulations of our model. This study will afford insights concerning assessment of mass drug administration, and the production of an accurate estimate of the possibility that the prevalence would resurge.

Materials and Methods

Parasite rate

The distribution of the parasite was surveyed in the 33 villages of the 11 islands in Vanuatu by the second author *et al.*. Blood samples were obtained from the 11,590 islanders. They reported age-specific prevalence of *P. falciparum, P. vivax, P. malariae*, mixed infection, and *P. falciparum* gametocyte (Kaneko *et al.*, 1994). On the other hand, United Nations (1992) published the demographic data of Vanuatu. By taking an average of the field data in Vanuatu weighted with the age specific ratio of population, we can estimate the overall parasite rates as follows.

P. falciparum	4.8%
P. vivax	5.2%
P. malariae	0.075%
P. falciparum gametocyte	0.98%

Vectorial capacity

It is well known that the principal species of mosquito vectors of malaria on the islands of Vanuatu is *Anopheles farauti*. The vectorial capacity that was introduced by Garrett-Jones (1964) has a synthetical characteristic of the infectiousness of the mosquito vector. It depends on the man-biting rate, the man-biting habit, the daily survival property in vectors, *etc.*. Therefore the vectorial capacity is much affected by the kind of species of mosquito. The vectorial capacity is formulated as follows:

$$C = ma^2p^n/(-\log p)$$

Here the parameters m, a, p, and n in the formula stand for the density of vectors in relation to man, the number of blood meals taken on man per vector per day, the daily survival probability of vectors, and the incubation period in vector, respectively.

The vectorial capacity can be reduced by designing an anti-malarial measure to control the cycle of transmission, the effect of which will last for a long time, whereas that of mass drug administration will last for a brief time. Actually, a malaria control project has made use of anti-malarial measures such as:

1) distribution of bed net
2) insecticide fogging
3) breeding fishes, which eat mosquito larvae in a stream or a pond
4) construction of drains not to propagate mosquitoes

The implementation of the measure 2), 3), or 4), which is a plan of the vector control, can decrease variable m in the vectorial capacity; on the other hand, that of the measure 1) can decrease a. If variable a can be reduced to 90%, the vectorial capacity will be lower to 80%, as the square of a is proportionate to C. A bed net is used to prevent a man from the contact with a vector in the night. Moreover the usage of a permethrin impregnated bed net could reduce both the density of vectors around an inhabitant and the survival probability of infectious mosquitoes (Charlwood and Graves, 1987; Kere *et al.*, 1993). For that reason, it will achieve the great effect of transmission blocking on a species of mosquito conveying malarial parasites which have a biting habit in the night that a biting peak comes at midnight, such as *Anopheles punctulatus*. When a man uses a bed net from 11:00 p.m. to 6:00 a.m., it can protect him about 73% from being bitten by vectors during the indoor night time from 5:00 p.m. to 8:00 a.m., based on previous observations in the Solomon Islands context (Ishii, 1993). For *Anopheles farauti* case, we can estimate about 50% protection in the same situation using-previous observations (Ishii, 1993). The second author *et al.* investigated the effect of the permethrin impregnated bed nets they had distributed in Maewo island in 1991. They reported the parasite rate of *P. falciparum* decreased to 1.5% after the distribution, though it held 10.6% before (Kaneko *et al.*, 1994). It is indeed difficult to estimate the degree of the diminution in the vectorial capacity in consequence of the enforcement of anti-malarial measures. Therefore, in Results and Discussion section, we have carried out several simulations of our malaria transmission model under the various conditions, for example, that the vectorial capacity is stable, or decreasing to 10%, 20%, 30%, 40% or 50%.

Transmission Blocking

To block the cycle of transmission of malarial parasites from an individual to a mosquito, a gametocidal drug will be dosed to all the villagers, although it cannot protect an individual against malaria infection. When primaquine against gametocytes is administered to the population, we have provided a rate ρ of intake (80% for example) in the population to reflect the real situation in the villages. Concerning schizonticidal drugs, which are administered simultaneously with primaquine, that is, chloroquine, and Fan-

sidar that is added only occasionally, we simply assume that the effective proportion equals to ρ. It was also reported (Kaneko *et al.*, 1994) that there were several villagers in Vanuatu who had drug resistant parasites, especially against chloroquine.

Malaria Transmission Model with Variable Vectorial Capacity

The previous malaria transmission models were composed on the assumption that the vectorial capacity was stable. But the stable model will produce an overestimated rate of relapse of malaria, as a certain antimalarial measure is used together with mass drug administration in a malaria control project. In the present paper, we have constructed a model in which the vectorial capacity begins gradually to decrease one month after vector control measures are effected, reaching a settled rate, for example, at 10% or 20%, of the diminution two months later, and holding the stable equilibrium after that. The fundamental structure is based on DMT model and Collett-Lye model developed from DMT model. To treat a malaria transmission model by difference equations, the human population is divided into the following ten classes; we use the same symbols for each classes as in Collett and Lye (1987):

Non-immune susceptive x_1
Non-immune incubating x_2
Immune susceptive x_3
Immune incubating x_4
Non-immune infectious positive y_1
Non-immune positive y_2
Immune positive y_3
Non-immune positive, non-infectious z_1
(resulting from the effect of a gametocidal drug)
Non-immune protected z_2
(resulting from the effect of a schizonticidal drug)
Immune protected z_3
(resulting from the effect of a schizonticidal drug)

The malaria transmission model involves several parameters that have to be determined by the entomological observations and the epidemiological studies. Some of them do not depend on a specific region much; the following two parameters n, N are adopted from the previous studies (Dietz *et al.*, 1974) such as:

the incubation period (days) in vector $n=10$
the incubation period (days) in man $N=15$

The effect of administration of a gametocidal drug transfers the individuals in y_1-class to z_1-class, and that of administration of a schizonticidal drug, in y_2-class and y_3-class to z_2-class and z_3-class, respectively. The parameter γ stands for the rate of transfer from z_1-class to y_1-class per day, and β stands for the rate of transfer from z_2-class and z_3-class to y_2-class and y_3-class per day after enforcement of mass drug administration. These parameters are given as follows:

$\beta=1-\exp(-1/d_1)$, $\gamma=1-\exp(-1/d_2)$

where d_1, d_2 denote the number of days of protection. For chloroquine and primaquine,

$d_1=10$, and $d_2=15$, then $\beta=0.09516$, $\gamma=0.06499$ (Collett and Lye, 1987)

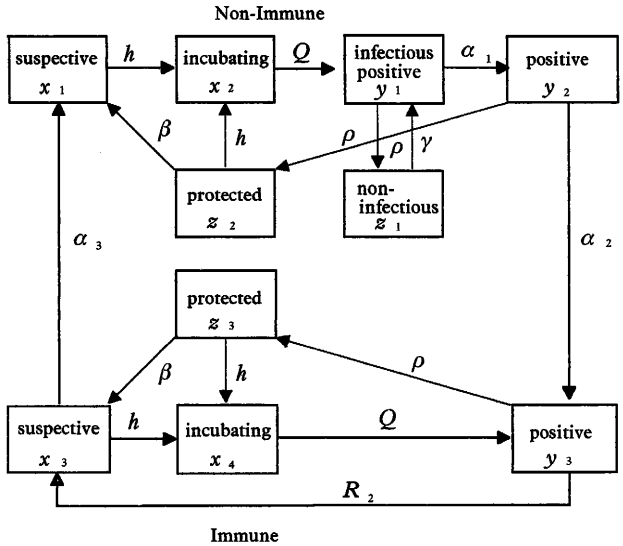


Figure 1. The scheme of our malaria transmission model showing the transfers between the 10 classes, the 6 parameters and the 3 functions *i.e.* $h(t)$, $Q(t)$, $R_2(t)$ which are defined in Appendix. It was originally proposed by Dietz *et al.* (1974) and modified by Collett and Lye (1987). The parameter α_3 is added to this scheme. δ is omitted from this figure.

For these drugs, we assume $\rho=0.8$, the value of which was used in Collett and Lye (1987). Meanwhile the vital statistics in Vanuatu (United Nations, 1992) give the parameter δ:

the birth rate per person per day $\delta=1.18\times10^{-4}$

In regard to the recovery rates, we have adopted the values listed in Collett and Lye (1987):

the recovery rate per day for non-immune individual $r_1=0.0$

the recovery rate per day for immune individual $r_2=0.0327$

Our model also uses the following parameters: the rate a_1 of loss of infectiousness per day, the rate a_2 of acquisition of immunity per day, and the susceptibility g of individual to infection. It is a peculiarity of the epidemicity of malaria in the Vanuatu endemic region that the decreasing rate of the age-specific prevalence is less than that of the estimation calculated by the previously proposed models. From an analytical point of view, we attribute the situation mentioned above to the loss of immunity. In order to fit the prevalence curve from the model to the distribution of the observational data, we have, therefore, incorporated a new parameter a_3, viz., the rate of loss of immunity per day into our model. Above four parameters will be considered in next section. The scheme of our malaria transmission model is shown in Figure 1, and the system of the nonlinear difference equations of our model is given in Appendix.

RESULTS AND DISCUSSION

FITING OF THE MODEL AND ESTIMATION OF PARAMETERS

In this subsection, we will determine the remaining parameters. For four parameters a_1, a_2, a_3, and g in our model, using the prevalence curve by age, we may choose them suitably so that the model provides a satisfactory fit to the distribution of the field data; the prevalence curve by age is obtained through our model simulation under the condition that $\delta=0$, and the initial value conditions $x_1=1.0$ and that the other variables are equal to 0. The fitted parameters are given as follows:

$a_1=0.0067$, $a_2=0.002$, $a_3=0.00002$, $g=0.012$

For the four fitted parameters, χ^2-valve is estimated at 5.02 on 20 data points. The fitted prevalence curve is shown in Figure 2.

The calculation of the equilibrium of the model has drawn the relation among the vectorial capacity, the parasite rate of *P. falciparum*, and *P. falciparum* gametocyte to curves in Figure 3, on the basis of a set of parameters determined in the previous subsection and this one. To get the relational curve, the simulations were performed for a long range at about 0.1 step C-values. Applying the estimated parasite rate of Vanuatu in Parasite rate subsection, the vectorial capacity C has been determined as follows:

$C=1.2$

Under the fitted parameters which are adopted in this subsection and the previous one, the initial values of the system of the difference equations for our model that are obtained through the state of equilibrium in our

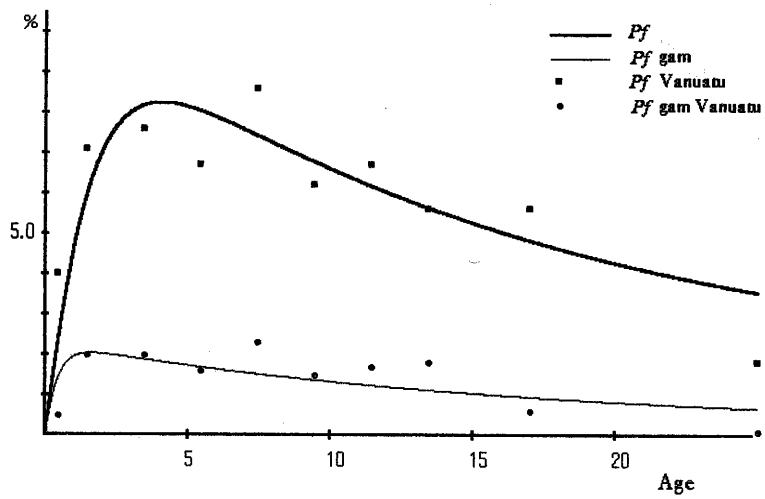


Figure 2. Comparison between the observational data in Vanuatu (Kaneko *et al.*, 1994) and the curves estimated by our model using the parameters given in this section with respect to the prevalence rate (%) of *P. falciparum* and that of *P. falciparum* gametocyte by age.

model are given as follows:

$(x_1, x_2, x_3, x_4)=(0.476, 0.001, 0.475, 0.0004)$

$(y_1, y_2, y_3)=(0.010, 0.033, 0.004)$

By the definition, the initial values of all z_is' are equal to $0(i=1, 2, 3)$.

Simulations of The Model

The mathematical model of malaria transmission with variable vectorial capacity value which is formed as the system of non-linear difference equations has been programmed by Turbo PASCAL TM.. It works on the Micro-Soft Windows TM. platform, and numerically solves a simulation with the iteration day by day. We have simulated more than fifty cases, including the uniform vectorial capacity-model, and the decreasing vectorial capacity-models at the rate of 10%, 20%, 30%, 40%, and 50%. Moreover, the simulations have been carried out in the cases that from zero to nine times-mass drug administration is executed. We assume that when mass drug administration is executed twice or more we estimate the rate of intake compliance at 80% except the case of the Aneityum context (see below No. 4). In this paper, we have inserted the several figures of the situations performed by our model for the typical cases.

The results of our simulations are described below.

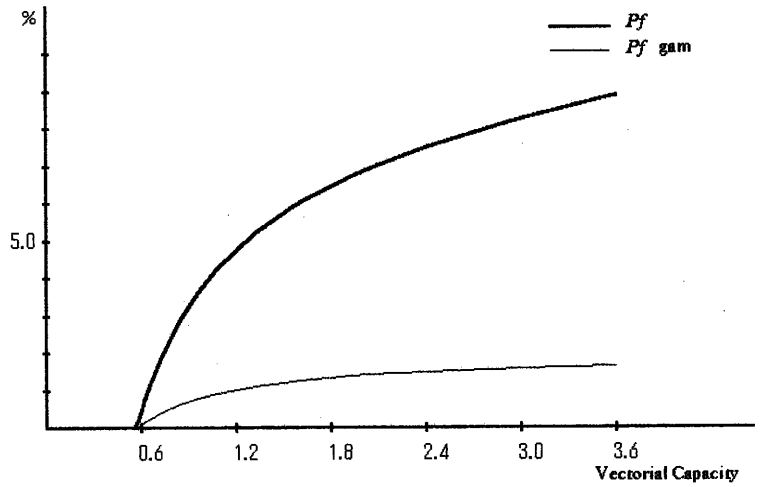


Figure 3. Relation among the vectorial capacity, the overall prevalence rate (%) of *P. falciparum* and of *P. falciparum* gametocyte, which is obtained through the simulation for a long range.

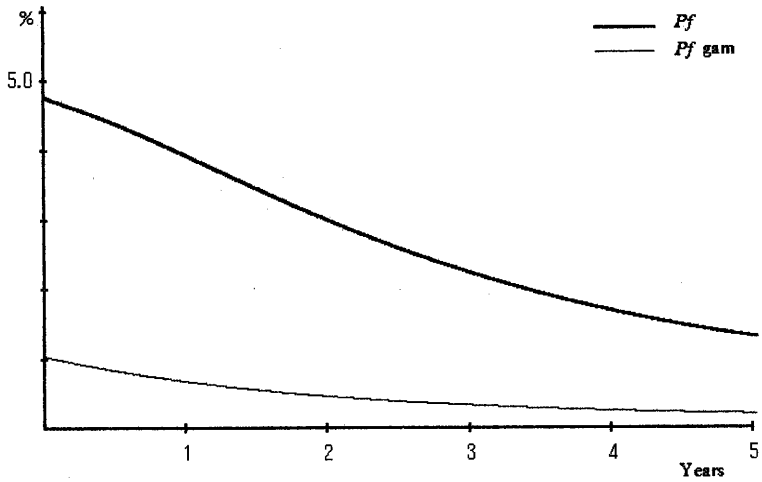


Figure 4. Variation of the overall prevalence rate (%) of *P. falciparum* and of *P. falciparum* gametocyte under the rate 100% of intake compliance, the vectorial capacity diminishing in the proportion of 10%.

1. The public health project aiming at decreasing the vectorial capacity, such as by the distribution of insecticide impregnated bed nets, is vitally important in that it can securely reduce the prevalence rate for a long range. If the vectorial capacity diminishes in the proportion of 10%, 20%, 30%, 40%, or 50%, the prevalence rate, the initial value of which is estimated as 4.8% reduces to 4.3%, 3.6%, 2.9%, 1.8%, or 0.5%, respectively (Fig. 3). Especially, if the vectorial capacity reduces by half, the prevalence of malaria tends toward an end. Figure 4 shows the simulation in the case that the vectorial capacity diminishes in the proportion of 20%.
2. We cannot expect a good effect on transmission blocking for a long range in the case in which mass drug administration, say MDA, is put into practice once or twice. Without the enforcement of anti-malarial measure, though the prevalence is decreasing abruptly after MDA, it is turning back at the level of beginning within 4 or 5 years, according to one time-MDA case or two times-MDA case. Also with the enforcement of anti-malarial measures, it is securely

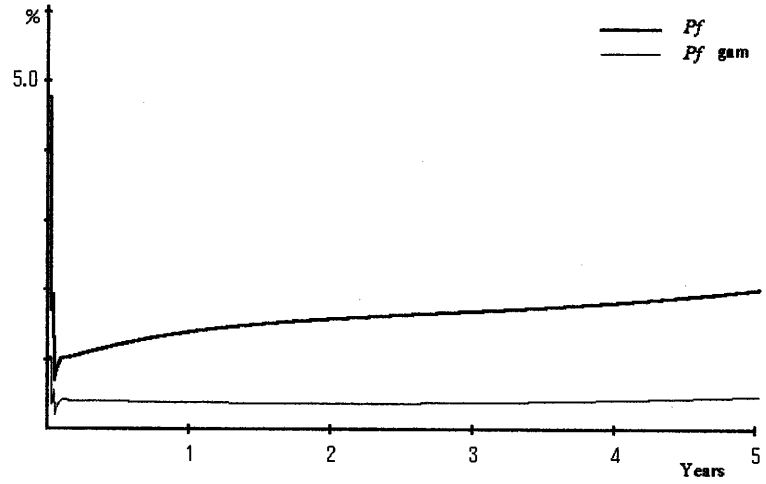


Figure 5. Variation of the overall prevalence rate (%) of *P. falciparum* and of *P. falciparum* gametocyte under the rate 80% of intake compliance, when two times-MDA is executed at an interval of one week, the vectorial capacity diminishing in the proportion of 10%.

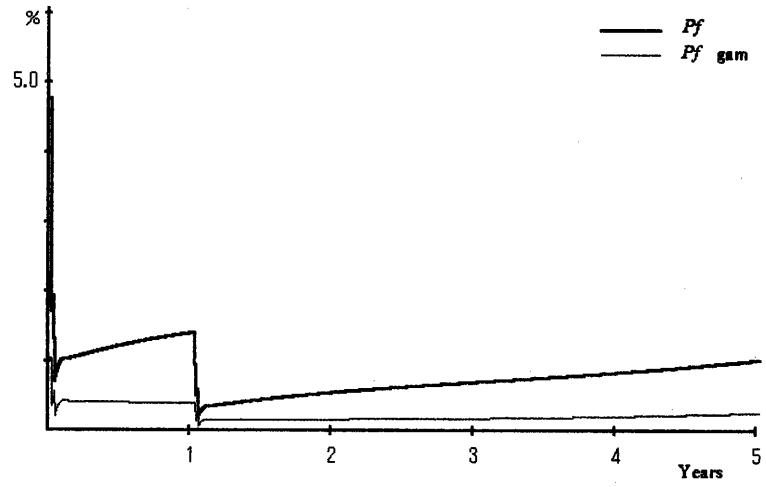


Figure 6. Variation of the overall prevalence rate (%) of *P. falciparum* and of *P. falciparum* gametocyte under the rate 80% of intake compliance, in the case that the execution of two times-MDA at an interval of one week is iterated twice at an interval of one year, when the vectorial capacity diminishing in the proportion of 10%.

approaching to the equilibrium point related to the current vectorial capacity in one or two times-MDA case. If the prevalence would not fairly decline by MDA, it would resurge before long. Figure 5 shows the simulation in the case of two times-MDA with 10% diminution in the vectorial capacity.

3. We simulate the case in which the execution of two times-MDA is iterated twice at an interval of one year, with the result that the total number of MDA comes to four times, under the condition that the vectorial capacity is decreasing at the rate of 10%, in Figure 6. This scheme will succeed in keeping the prevalence at low level for a few years, but we cannot conclude that it will become the final settlement in the long-range eradication of malaria prevalence.

4. The second author *et al.* carried out a malaria control program in Aneityum Island in 1991 in which MDA consisting of primaquine, chloroquine, and Fansidar (only three times) was executed nine times at an interval of a week for a proportion at 88% of all the islanders just before a wet season, and in which the permethrin impregnated bed nets were distributed to

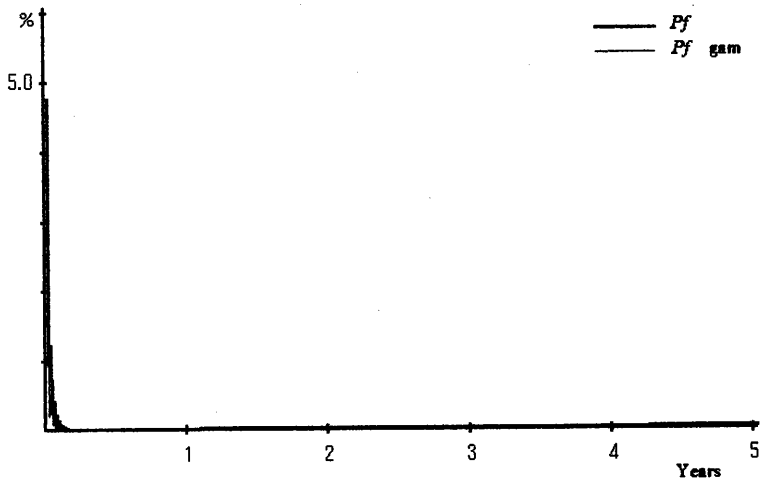


Figure 7. Variation of the overall prevalence rate (%) of *P. falciparum* and of *P. falciparum* gametocyte under the rate 88% of intake compliance, when nine times-MDA is executed at an interval of one week, the vectorial capacity diminishing in the proportion of 10%. This graph shows the result of the simulation in the Aneityum context (Kaneko *et al.*, 1994).

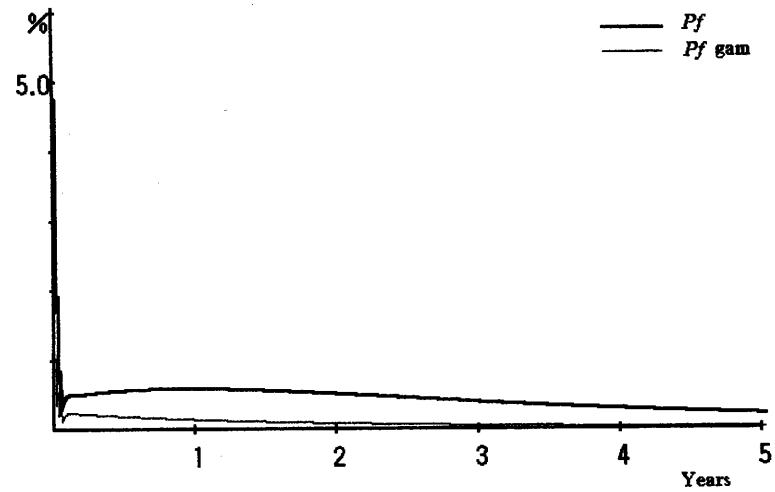


Figure 8. Variation of the overall prevalence rate (%) of *P. falciparum* and of *P. falciparum* gametocyte under the rate 80% of intake compliance, when three times-MDA is executed at an interval of one week, the vectorial capacity diminishing in the proportion of 30%.

almost all islanders (Kaneko *et al.*, 1994). We assume that the distribution of the bed nets reduces the vectorial capacity to 80%-90% of its beginning. Figure 7 shows the result of the simulation in the Aneityum context. It seems that the resurgence of malaria will not occur for a long time by the thorough execution of MDA. Nevertheless, there are indications of the resurgence in the situation in which the number of MDA is less than nine (7 or 8 times). Moreover, in the case of nine times-MDA without the enforcement of anti-malarial measures, the simulation which is not printed here shows that the same circumstances will occur as above.

5. When the vectorial capacity diminishes in the proportion of 30% or more by the execution of anti-malarial measures, the trial of three times-MDA makes the prevalence rate reduced to less than 0.5% (Fig. 8); moreover the trial of five times-MDA makes the prevalence of malaria tending toward the long-range eradication. If we can hold the level of the vectorial capacity half as much as that of beginning, the prevalence of malaria is going to decline and the resurgence will seldom occur for a long time. In this case, the execution of two or three times-MDA also furthers the reduction of malaria prevalence rapidly. We think that even when a sporadic infection may occur in the control region or an infectious individual may trespass into that region, the execution of anti-malarial measures will be effective from a point of view of prevention against the epidemic of malaria.

ACKNOWLEDGMENTS

This research was partially supported by Grant-in-Aid for Science Research, the Ministry of Health and Welfare: No. 06041135, A. Ishii (the principal investigator), Research on malaria and related hemotological disorders in south Pacific region.

APPENDIX

The system of the difference equations of our model is given in this appendix. All the parameters appeared in these equations are defined in Transmission blocking, Malaria transmission model with Variable vectorial capacity, and Fitting of the model and estimation of parameters subsections.

Equations

$$\Delta x_1=\delta-(h(t)+\delta)x_1(t)+\beta(z_1(t)+z_2(t))+\alpha_3 x_3(t)$$
$$\Delta x_2=h(t)x_1(t)-Q(t)x_1(t-N)-\delta x_2(t)+h(t)(z_1(t)+z_2(t))$$
$$\Delta x_3=R_2(t)y_3(t)-(h(t)+\delta)x_3(t)+\beta z_3(t)-\alpha_3 x_3(t)$$
$$\Delta x_4=h(t)x_3(t)-Q(t)x_3(t-N)-\delta x_4(t)+h(t)z_3(t)$$
$$\Delta y_1=Q(t)x_1(t-N)-(\alpha_1+\delta)y_1(t)+\gamma z_1(t)$$
$$\Delta y_2=\alpha_1 y_1(t)-(\alpha_2+\delta)y_2(t)$$
$$\Delta y_3=\alpha_2 y_2(t)+Q(t)x_3(t-N)-(R_2(t)+\delta)y_3(t)$$
$$\Delta z_1=-(\beta+\delta+h(t)+\gamma)z_1(t)$$
$$\Delta z_2=-(\beta+\delta+h(t))z_2(t)$$
$$\Delta z_3=-(\beta+\delta+h(t))z_3(t)$$

When MDA (gametocidal drugs) is put into practice at t_1, in the following equations, each term of the left hand side is replaced immediately with that of right hand side:

$$z_1(t)=\rho y_1(t_1),\ y_1(t)=(1-\rho)y_1(t_1)$$

Also, when MDA (schizonticidal drugs) is put into practice at t_1, in the following equations, each term of the left hand side is replaced immediately with that of right hand side:

$$z_i(t)=\rho y_i(t_1),\ y_i(t)=(1-\rho)y_i(t_1),\ (i=2, 3)$$

Notations

Δ denotes the difference operator, *i.e.*, $\Delta f=f(t+1)-f(t)$ for any function f.

The daily rate $h(t)$ at which susceptible class (x_1, x_3) is transferred to positive class (y_2, y_3) through the incubating class (x_2, x_4) is expressed by:

$$h(t)=g(1-\exp(-C(t)y_1(t-n))$$

Put:

$$Q(t)=(1-\delta)^N\ h(t-N)$$

The recovery rate $R_2(t)$ of y_3-class is given in the following formula:

$$R_2(t)=h(t)/(\exp(h(t)/r_2)-1)$$

REFERENCES

1) Charlwood, J.D. and Graves, P.M. (1987): The effect of permethrin-impregnated bednets on a population of *Anopheles farauti* in coastal Papua New Guinea, Med. Vet. Entomol., 1, 319-327

2) Collett, D. and Lye, M.S. (1987): Modeling the effect of intervention on the transmission of Malaria in East Malaysia, Statist. Med., 6, 853-861

3) Department of International Economics and Social Affairs, Statistical office, United Nations (1992): Demographic Year book, 1990, United Nations, New York

4) Dietz, K., Molineaux, L. and Thomas, A. (1974): A Malaria model tested in the African Savannah, Bull. WHO, 50, 347-357

5) Garrett-Jones, C. (1964): The human blood index of malaria vectors in relation to epidemiological assessment, Bull. WHO, 30, 241-261

6) Ishii, A. (1993): Parasitology and countermeasure of malaria on Solomon Islands, Nettai (Tropics), Japanese Association Tropical Medicine, 26, 195-207

7) Kaneko, A., Taleo, G.K. and Rieckmann, K.H. (1994):

Island malaria control in eastern Melanesia: 1) Malaria eliminated from a small island by 9-week mass drug administration and impregnated bednets, Japan. J. Parasitol., 43, 358-370

8) Kaneko, A., Taleo, G.K. and Shirakawa, H. (1994): Island malaria control in eastern Melanesia: 2) Age-specific manifestation of drug resistance of *Plasmodium falciparum* and *Plasmodium vivax* in islanders, ibid., 43, 371-383

9) Kere, N.K., Parkinson, A.D. and Samramickerema, W. A. (1993): The effect of permethrin impregnated bednets on the incidence of *Plasmodium falciparum*, in children of north Guadalcanal, Solomon Islands, Southeast Asian, J. Trop. Med. Publ. Hlth., 24, 130-137

Jpn. J. Trop. Med. Hyg., Vol. 24, No. 1, 1996, pp. 21-26

EFFECT OF INDOOR-KEEPING OF HOUSE-DOGS ON THE TRANSMISSION OF *DIROFILARIA IMMITIS* IN NAGASAKI CITY, JAPAN

TSUTOMU ODA[1], MARIKO MINE[2], OSAMU SUENAGA[3], MAKOTO ZAITSU[4], KENJI KUROKAWA[5], KOICHIRO FUJITA[6], KATSUTOMO KATO[7], YASUNORI OGAWA[8] AND ICHIRO YAMAZAKI[8]

Received November 28, 1995/Accepted January 9, 1996

Abstract: The positive rate of house-dogs for *Dirofilaria immitis* microfilariae decreased in 27 years from 1968 to 1994 in northern and southern parts of Nagasaki City. It was examined whether or not the increase of indoor-kept dogs contributed greatly to the reduction in positive rate of house-dogs in the city. Positive rate of dogs was generally lower in indoor-kept dogs than in outdoor-kept dogs. From the lower positive rate of indoor-kept dogs, *D. immitis* infection is considered to have occurred mainly in outdoor-kept dogs. Positive rate was high from 1968 to 1983 when rate of indoor-kept dogs was low and thereafter positive rate gradually decreased though the rate of indoor-kept dogs was not greatly changed. From this, increase of indoor-kept dogs may be a factor causing the decrease of positive rate in all examined dogs from 1968 to 1983, but the reduction of positive rate thereafter is considered to be due to decrease of positive rate in outdoor-kept dogs.

Keywords: *Dirofilaria immitis*, house-dogs, transmission, indoor-keeping, positive rate of microfilariae

INTRODUCTION

The prevalence of dirofilariasis in house-dogs decreased in 27 years from 1968 to 1994 in Nagasaki City, and the population of the main vector mosquito, *Culex pipiens pallens*, also decreased in parallel with the expansion of the sewage system (Oda *et al.*, 1994a: 1995). In addition, the results of a questionnaire survey on living environments of dogs (Oda *et al.*, 1994b) indicated the increase of households that kept dogs indoors. However, it was not clear whether or not the increase of indoor-kept dogs contributed greatly to the reduction in positive rate of dogs in the city. On the other hand, the infection with *Dirofilaria immitis* was reported in dogs kept indoors (Yasuda *et al.*, 1985). In this study we analyzed the role of indoor-kept dogs in the transmission of *D. immitis*.

STUDY AREA AND METHODS

To examine the microfilariae of *D. immitis*, we sampled blood from an earlobe of about 400 registered dogs in April or May once in each year from 1983 to 1994 in a southern district (Tomachi) and three northern districts (Sakamoto, Takao and Yamazato) in Nagasaki City. Blood samples were subjected to Giemsa staining. The data on breed, sex and age of examined dogs were taken from the record of Nagasaki City Health Center (Oda *et al.*, 1993; 1995). In addition, a questionnaire survey was conducted to owners of registered dogs

[1]Department of General Education, the School of Allied Medical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852, Japan
[2]Scientific Data Center of Atomic Bomb Disaster, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan
[3]Reference Center, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852, Japan
[4]Sasebo City Health Center, 1-10 Yahata-machi, Sasebo 857, Japan
[5]Department of Bacteriology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan
[6]Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan
[7]Department of Physical Therapy, the School of Allied Medical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852, Japan
[8]Nagasaki City Health Center, 2-34 Mori-machi, Nagasaki 852, Japan

in the northern and southern parts in 1989 and 1993 to know the living environment of domestic dogs, including a question whether they are kept indoors or outdoors. The details of the questionnaire survey method were reported in the previous paper (Oda *et al.*, 1994b). The breed names of dogs followed the book "Inu" (Ohno, 1973).

RESULTS

1. Breed of dogs kept indoors and outdoors

We carried out a questionnaire survey on living environments of house-dogs in 1989 and 1993, and based on the replies from dog owners, all dogs examined for *D. immitis* infection in each year were classified into dogs kept indoors (indoor group) and outdoors (outdoor group) (Table 1).

Among 370 dogs examined in Nagasaki City, 111 dogs were kept indoors, and the remaining 259 were kept outdoors. There were 15 breeds in the indoor group and 19 breeds in the outdoor group. Five breeds of Mixed breed (No. 1), Shiba Inu (No. 2), Shetland Sheepdog (No. 4), Beagle (No. 5) and Dalmatian (No. 14) were kept both indoors and outdoors.

2. Positive rate of *D. immitis* by dog breed

Table 2 shows the prevalence of *D. immitis* in dogs kept indoors and outdoors by dog breed based on the record of blood examination in the years from 1968 to 1994. Here, the dogs of 5 breeds, Nos. 1, 2, 4, 5 and 14 in the indoor group in Table 1, were regarded as those kept outdoors, because much more dogs were found in

Table 1 Breed of dogs kept indoors and outdoors in Nagasaki City, according to questionnaire

Dog breed		Indoor-kept dogs	Outdoor-kept dogs	Total
No.	Name	No. examined	No. examined	No. examined
1 *	Mixed Breed	34	170	204
2 *	Shiba Inu	5	42	47
3	Maltese	23		23
4 *	Shetland Sheepdog	8	8	16
5 *	Beagle	2	13	15
6	Yorkshire Terrier	9		9
7	Pomeranian	7		7
8	Chihuahua	5		5
9	Toy Poodle	5		5
10	Shih Tzu	4		4
11	Terrier		4	4
12	Dachshund	3		3
13	Pug	3		3
14*	Dalmatian	1	1	2
15	Setter		2	2
16	Afghan Hound		2	2
17	Bulldog		2	2
18	Japanese Spaniel		2	2
19	Pointer		2	2
20	Siberian Husky		2	2
21	Shikoku Inu		2	2
22	Wirehaired Fox Terrier		2	2
23	Papillon	1		1
24	Pekingese	1		1
25	Japanese Spitz		1	1
26	Akita Inu		1	1
27	American Cocker Spaniel		1	1
28	Doberman Pinscher		1	1
29	Labrador Retriever		1	1
Total		111	259	370

*Kept both indoors and outdoors.

Table 2 Microfilarial prevalence of *Dirofilaria immitis* by dog breed

Dog breed		Indoor-kept dogs			Outdoor-kept dogs			Total		
No.	Name	No. examined	No. positive	(%)	No. examined	No. positive	(%)	No. examined	No. positive	(%)
1	Mixed Breed				2979	806	27.1	2979	806	27.1
2	Shiba Inu				561	104	18.5	561	104	18.5
3	Shetland Sheepdog				254	27	10.6	254	27	10.6
4	Maltese	243	5	2.1				243	5	2.1
5	Beagle				158	34	21.5	158	34	21.5
6	Toy Poodle	110	1	0.9				110	0.6	0.9
7	Pomeranian	105	3	2.9				105	3	2.9
8	Yorkshire Terrier	104	0	0.0				104	0	0.0
9	Japanese Spitz				77	23	29.9	77	23	29.9
10	Terrier				47	10	21.3	47	10	21.3
11	Akita Inu				42	14	33.3	42	14	33.3
12	Shih Tzu	41	0	0.0				41	0	0.0
13	Dachshund	40	4	10.0				40	4	10.0
14	Chihuahua	38	0	0.0				38	0	0.0
15	Pug	32	1	3.1				32	1	3.1
16	Pointer				26	12	46.2	26	12	46.2
17	Chow Chow				20	3	15.0	20	3	15.0
18	Japanese Spaniel				20	1	5.0	20	1	5.0
19	German Shepherd Dog				19	7	36.8	19	7	36.8
20	Collie				17	5	29.4	17	5	29.4
21	Pekingese	16	3	18.8				16	3	18.8
22	Afghan Hound				16	0	0.0	16	0	0.0
23	Dalmatian				13	2	15.4	13	2	15.4
24	American Cocker Spaniel				13	4	30.8	13	4	30.8
25	Wirehaired Fox Terrier				13	1	7.7	13	1	7.7
26	Doberman Pinscher				13	4	30.8	13	4	30.8
27	Siberian Husky				11	0	0.0	11	0	0.0
28	Shikoku Inu				10	1	10.0	10	1	10.0
29	Labrador Retriever				9	0	0.0	9	0	0.0
30	Bulldog				9	2	22.2	9	2	22.2
31	Papillon	8	0	0.0				8	0	0.0
32	Setter				8	1	12.5	8	1	12.5
33	Scottish Terrier				6	1	16.7	6	1	16.7
34	Boxer				6	4	66.7	6	4	66.7
35	White Terrier				5	0	0.0	5	0	0.0
36	Kai Inu				4	1	25.0	4	1	25.0
37	Miniature Pinscher	3	0	0.0				3	0	0.0
38	Cavalier King Charles Spaniel				3	0	0.0	3	0	0.0
39	Golden Retriever				3	0	0.0	3	0	0.0
40	Kishu Inu				3	0	0.0	3	0	0.0
41	Tosa Inu				2	0	0.0	2	0	0.0
42	Boston Terrier				2	0	0.0	2	0	0.0
43	Mikawa Inu				1	0	0.0	1	0	0.0
44	Fox Terrier Smooth				1	0	0.0	1	0	0.0
45	St. Bernard				1	0	0.0	1	0	0.0
Total		740	17	2.3*	4372	1067	24.4*	5112**	1084	21.2

*Significant ($P<0.01$)

**This figure shows total number of house-dogs with blood examination and the clear record on breed name in period from 1968 to 1994 in Nagasaki City.

the outdoor group than in the indoor group. Thus, the indoor group consists of the following 11 breeds: Maltese, Yorkshire Terrier, Pomeranian, Toy Poodle, Dachshund, Shih Tzu, Pug, Chihuahua, Papillon, Miniature Pinscher and Pekingese. Miniature Pinscher was also added in the indoor-kept dog, because this was usually kept inside the house as toy dog, although not shown in a questionnaire survey.

The positive rate was 2.3% in the outdoor group but much higher (24.4%) in the outdoor group. Positive rate varied with dog breed in the indoor group as well as in the outdoor group.

3. Positive rates of dogs kept indoors and outdoors

Table 3 shows annual changes in the positive rates for microfilariae in indoor-kept and outdoor-kept dogs in each year from 1968 to 1994. The positive rate of indoor-kept dogs was much lower in any study year than that of outdoor-kept dogs. The positive rate of indoor-kept dogs was generally low from 1968 to 1986

Table 3 Annual changes in microfilarial prevalenece of *Dirofilaria immitis* in the dogs kept indoors or outdoors in Nagasaki City

Year	Indoor-kept dogs			Outdoor-kept dogs			Total			Percentage of Indoor-kept dogs {A/(A+B) ×100}
	No. examined (A)	No. positive	(%)	No. examined (B)	No. positive	(%)	No. examined (A+B)	No. positive	(%)	
1968	1	0	0.0	301	131	43.5	302	131	43.4	0.3
1977	23	5	21.7	214	79	36.9	237	84	35.4	9.7
1983	52	3	5.8	287	95	33.1	339	98	28.9	15.3
1984	66	4	6.1	338	100	29.6	404	104	25.7	16.3
1985	43	1	2.3	250	82	32.8	293	83	28.3	14.7
1986	24	1	4.2	277	78	28.2	301	79	26.2	8.0
1987	44	0	0.0	317	82	25.9	361	82	22.7	12.2
1988	64	0	0.0	387	98	25.3	451	98	21.7	14.2
1989	73	1	1.4	334	87	26.0	407	88	21.6	17.9
1990	80	0	0.0	342	61	17.8	422	61	14.5	19.0
1991	70	0	0.0	294	40	13.6	364	40	11.0	19.2
1992	63	1	1.6	349	55	15.8	412	56	13.6	15.3
1993	71	1	1.4	325	40	12.3	396	41	10.4	17.9
1994	66	0	0.0	357	39	10.9	423	39	9.2	15.6
Total	740	17	2.3	4372	1067	24.4	5112	1084	21.2	14.5

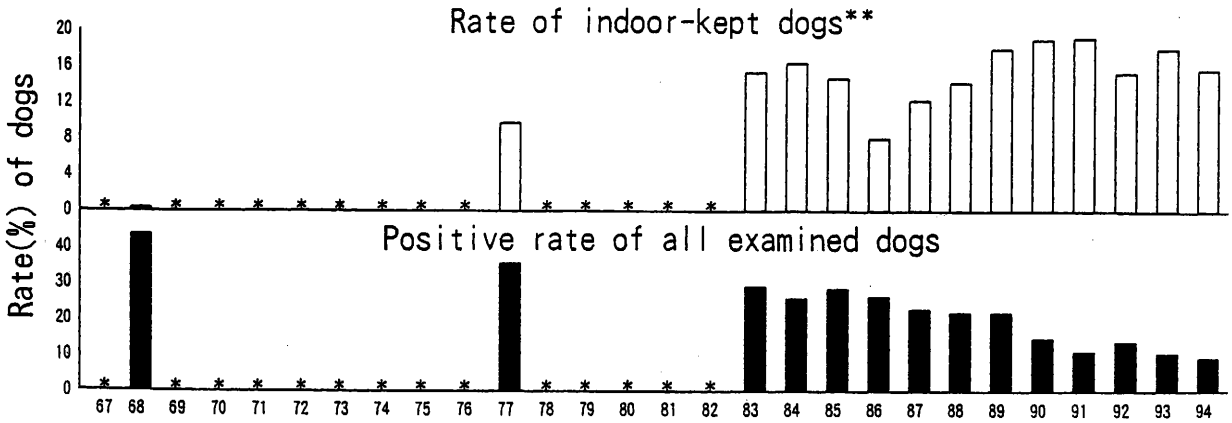


Figure 1 Annual changes in microfilarial prevalence of *Dirofilaria immitis* in the all examined dogs and rate of indoor-kept dogs in Nagasaki City.
*Data not available
**Rate of indoor-kept dogs shows percentage of, indoor-kept dogs for all examined dogs, A/(A+B) ×100, in Table 3.

and thereafter it became lower. The annual changes in positive rate were analyzed for all examined and outdoor-kept dogs by the trend test (Armitage, 1955). A decrease was observed in both rates from 1968 to 1994 ($p<0.01$).

4. Relationship between positive rate of all examined dogs and the rate of indoor-kept dogs

We calculated the rate of indoor-kept dogs for total number of examined dogs from Table 3 and showed annual changes in the positive rate of all examined dogs and the rate of indoor-kept dogs in Fig. 1, to make clear the relation between both rates. This figure indicated that the positive rate in all examined dogs was high when rate of indoor-kept dogs was low from 1968 to 1983 and thereafter the positive rate gradually decreased though rates of indoor-kept dogs were not greatly changed. Therefore, the reduction of positive rate after 1983 is considered to be due to decrease of positive rate in outdoor-kept dogs.

DISCUSSION

The present study clearly showed that the positive rate for *D. immitis* microfilariae was lower in indoor-kept dogs than in outdoor-kept dogs. This finding was consistent with the results reported by Appleton and Arlian (1979) and Thrasher *et al.* (1963: 1968). Wada *et al.* (1989) reported that the protection of humans from mosquito bites increased in Nagasaki City. This really means that it became difficult for mosquitoes to enter a house with closed windows due to recent widespread use of air conditioners. This may be the primary cause for the lower positive rate in the indoor-kept dogs.

As described above, the population of the indoor-kept dogs accounted for about 15% of all examined dogs and the positive rate was markedly lower in indoor-kept dogs than in outdoor-kept dogs. In other words, these results suggest that *D. immitis* infection occurs mainly in house-dogs kept outdoors. From present and previous results, the decrease in *Cx. p. pallens* seems to play an important role in the decrease of the positive rate in house-dogs in Nagasaki City. The increase of indoor-kept dogs may be a factor for the decrease of the positive rate in 1968 to 1983, and thereafter, the reduction of positive rate is considered to be due to the decrease of positive rate in outdoor-kept dogs. Other possible causes are the use of repellent coils and preventive drugs such as Ivermectin, but their effects are estimated to be not high, as suggested by Oda *et al.* (1995). In addition, change in the composition of dog breed may have a role for reduction in positive rate, but in our data, positive rate did not vary by the breed. The dogs with long hair is supposed to be low in positive rate, but Ohishi (1986) reported that positive rates were not different between dogs with long hair and those with short hair.

ACKNOWLEDGMENTS

Professor Y. Wada, Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University kindly read this manuscript and provided detailed suggestions. Dr. T. Fukuda, President, and members of the Society of Veterinarians in Nagasaki City really cooperated with our research. We wish to express our sincere gratitude to these persons.

REFERENCES

1) Armitage, P. (1955): Test for linear trends in proportions and frequencies. Biometrics 11, 375-386
2) Appleton, G.L. and Arlian, L.G. (1979): Canine filariasis in southwestern Ohio. OHIO J. Sci. 79(3): 136-138
3) Oda, T., Suenage, O., Mori, A., Fujita, K., Zaitsu, M., Kurokawa, K., Nishioka, T., Itoh, T., and Mine, M. (1993): Studies on epidemiology of *Dirofilaria immitis* in house dogs in Nagasaki City, Japan, with considerations on yearly changes in microfilarial prevalence. Jpn. J. Trop. Med. Hyg., 21, 231-237
4) Oda, T., Suenaga, O., Fujita, K., Zaitsu, M., Kurokawa, K., Ogawa, Y., Yamazaki, I., Iida, K., and Mine, M. (1994a): Comparison of population of vector mosquitoes of *Dirofilaria immitis* and their natural infection rates in southern and northern parts of Nagasaki City, Japan. Jpn. J. Trop. Med. Hyg., 22, 196-202
5) Oda, T., Suenaga, O., and Mine, M. (1994b): A questionnaire survey of living environments of house dogs and measures taken by dog owners to prevent zoonotic parasite, *Dirofilaria immitis* infection in Nagasaki City, Japan. Bull. Sch. Allied. Med. Sci., Nagasaki Univ., 8, 9-16
6) Oda, T., Suenaga, O., Zaitsu, M., Mori A., Kurokawa, K., Fujita K., Ogawa, Y., Yamazaki, I., Iida, K., Doi, K., Mine, M., and Kato, K. (1995): Studies on annual changes in microfilarial prevalence of *Dirofilaria immitis* among house dogs for 27 years in Nagasaki City, Japan. J. Trop. Med. Hyg., 23, 133-137
7) Ohishi, I. (1986): Inushijochu, -kiseichugakunotachibakara-, 136, Bun-eido, Tokyo (in Japanese).
8) Ohno, J. (1973): Inu, 1-230, Hoikusha, Tokyo (in Japanese).
9) Thrasher, J.P., Ash, L.R. and Little, M.D. (1963): Filarial infections of dogs in New Orleans. J.A.V.M.A., 143 (6), 605-608

10) Thrasher, J.P. and Clanton, J.R. (1968): Epizootiologic observations of canine filariasis in Georgia. J.A.V.M.A. 152 (10) : 1517-1520
11) Wada, Y., Tsuda, Y., and Suenaga, O. (1989): Transmission dynamics of *Dirofilaria immitis* in a southwestern part of Japan. Trop. Med., 31, 35-47
12) Yasuda, H., Kawamura, N., Yamamoto, K., and Zhan, Y. (1989): Studies on the microfilarial prevalence among house dogs in Sapporo City, July 1988-August 1989. J. Hokkaido Vet. Med. Assoc., 33, 319-321

Jpn. J. Trop. Med. Hyg., Vol. 24, No. 1, 1996, pp. 27-33

総　説

メフロキンによるマラリア治療の現況

木 村 幹 男*

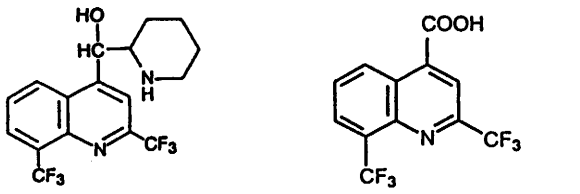
平成7年11月30日受付/平成8年1月11日受理

1．経　緯

1960年前後に東南アジア（タイ）と南米（コロンビア）において，クロロキン耐性熱帯熱マラリアが出現し，それが世界各地に急速に拡がったことから，新しい抗マラリア薬が待望されていた。米国Walter Reed陸軍医学研究所において抗マラリア薬のスクリーニングが精力的に行われ，その結果，ベトナム戦争の最中の1971年に約23万の物質の中から強力な作用を持つものとして，メフロキンが浮上した。抗原虫作用，薬理作用などの検討とともに，1976年からはWHO-TDRの計画に組み込まれ，広範な臨床試験の結果から効果，副作用の面で有望との結論が出た。その後，販売権がF. Hoffmann-La Roche社（以下Roche社）に譲渡され，Lariam®の商品名で1985年にはスイス，1986年にフランス，1987年にオーストラリアとドイツにおいて認可・発売がなされ，その後続々と各国でも使用される様になり，今では熱帯熱マラリアの第一選択薬剤としての地位を確立した。また，Mepha社はメフロキンの最高血中濃度の上昇を抑える目的で，特殊加工した製剤であるMephaquine Lactab®を開発し，これも市場に出回っている。特許が切れたことから，さらに他のメフロキン製剤も出始めている。

2．構造，作用

メフロキンは通常塩酸メフロキンとして用いられるが，化学名はDL-erythro-α-(2-piperidyl)-2,8-bis (trifluromethyl)-4-quinoline methanol hydrochlorideであり，キニーネと共に4-キノリンメタノール誘導体に分類される（図1）。キノリン核を有する点ではクロロキン，プリマキンなどとも共通である。分子量は塩酸メフロキンで414.8，メフロキン塩基で378.3である。単にメフロキンと言った場合，メフロキン塩基としての量を指すことに注意を要する。メフロキンが作用するのは赤内型無性原虫であり，その中では輪状体よりも成熟栄養体や分裂体のステージに良く作用する。肝細胞内休眠原虫や熱帯熱マラリア原虫の生殖母体には作用しない。作用機序はキニーネ，クロロキンなどの場合と同様であると考えられているが，確立したわけではなく，以下の可能性が挙げられている。1）原虫はヒト赤血球のヘモグロビンをその酸性食胞内で消化し，フェリプロトポルフィリンIX（ヘム）を形成するが，これは原虫膜に対する傷害性，酵素の阻害作用を有する。通常は，このヘムをヘムポリメラーゼによってタンパク結合させ，マラリア色素である無毒なヘモゾインへと変換する。メフロキンは食胞内に蓄積してヘム・メフロキン結合物を形成し，ヘムのタンパク結合を阻害して原虫に傷害性に働く。2）原虫DNAとインターカレーションして，その合成を阻害する。3）メフロキンの弱塩基作用により，通常は酸性であるべき食胞のpHを上昇させ，ヘモグロビンの消化を阻害する。



Mefloquine　　Carboxylic acid metabolite

Figure 1 Structural formulae of mefloquine and its carboxylic acid metabolite.

3．薬物動態

健康人での腸管からの吸収は早く，吸収半減期は1-4（平均2.1）時間であり，最高血中濃度到達時間は6-24（平均17.6）時間である。血中半減期は比較的長く14-28（平均18.1）日である。食事とともに服用することで吸収は高まる。マラリア患者では，吸収半減期が5-6（平均5.4）時間と長くなり，最高血中濃度到達時間は12-23（平均16.6）時間で余り変わらないが，その濃度は高くなる傾向がある。血中半減期は10-15（平均12.3）日と短縮する（Palmer *et al.*, 1993）。血中では98%が血漿タンパクと結合し，赤血球膜との結合性が高いため，赤血球内濃度は血漿中濃度よりも高い。代謝物としてカルボキシル酸体が生成されるが（図1），これには抗原虫活性はない。未変化体，代謝物ともに

*東京大学医科学研究所感染免疫内科

胆汁から便へと排泄されるが，腸肝循環もある。幼児における体内動態は成人と殆ど同じであり，妊婦では血中濃度がやや低く非妊婦の7-8割程度である（Na Bangchang *et al.*, 1994)。乳汁への分泌はあるが，最高乳汁中濃度は最高血漿中濃度に比して10分の1程度であり，投与後4日間での乳汁中濃度/血漿中濃度でみても0.13-0.16と低い（Edstein *et al.*, 1988)。体内動態には人種差もあると言われており，例えばアジア人と欧米人を比べた場合，前者の方が最高血中濃度が2倍以上高くなるとのデータもある(Palmer *et al.*, 1993)。この問題は今後さらに広範囲に検討されなければならない。

4．用量・用法

Roche社ではnon-immuneで体重≥60kgの場合1,500mg，<60kgの場合1,250mgとし，semi-immuneでは体重≥60kgの場合1,000mg，<60kgの場合750mgとしている。3錠(750mg)までは単回投与で良いが，それを超す場合には6-8時間間隔で2-3回の分割投与とする。これに対して，WHOでは免疫状態の有無にかかわらず，1,000mgか15mg/kgのうち少ない方の量を単回投与としている（WHO, 1994)。

当院においては初めの時期はRoche社の用法・用量に従っていたが，最近では免疫状態の有無にかかわらずそのsemi-immuneでの投与量を採用し，低用量での治療を行っている。

5．臨床効果

認可・発売前の1980～1982年に行われた用量設定の治験において，今では薬剤耐性の問題が深刻なタイ，ミャンマーにおいても，500～1,500mgの範囲の投与量で殆どが90%以上の治癒率が得られている（Palmer *et al.*, 1993)。最近報告されたいくつかの国での臨床効果を表1に示す。セネガル（Hatin *et al.*, 1992)，ナイジェリア（Sowunmi *et al.*, 1992)などの西アフリカ地域においては，今でも100%近い治癒率が得られている。マラウイ（Slutsker *et al.*, 1990)，ナイジェリア（Oduola *et al.*, 1992）などからRI型再燃を思わせる例の報告はあるが，それらの地域では再感染を否定できない。マラリア非流行地たとえばデンマークでは，55人のデンマーク人と22人のアフリカ出身デンマーク居住者を含む計81人につきメフロキン1,500mgの投与を行ったところ，RI再燃の1例を除き，他の80例は全て治癒している（Lariam® Product Monograph, 1992）（表1)。世界の様々な地域からin vitro感受性試験におけるメフロキン低感受性・耐性が報告されてはいるが，in vivoでの効果と合わないことも多く，例えば，in vitroで低感受性を示した熱帯熱マラリア7例が全てメフロキン12.5mg/kgの投与で治癒しているし（Hatin *et al.*, 1992)，同様な報告は他にも見られる（Sowunmi *et al.*, 1992)。しかし今後は，各地においてin vivoでの治療失敗例が増える可能性を念頭に置かなければならない。

東南アジアの特定地域ではメフロキンによる治療が成功しないことが多い。例えば，タイ・カンボジアの国境地域での熱帯熱マラリアに対してメフロキン15mg/kgの治療を行った結果では，第42日で判定しての治療失敗率が59.1%にのぼり，内訳はRIが31.8%，RIIが20.9%，RIIIが6.4%であった（Fontanet *et al.*, 1993)。同じ地域の他の報告では，15mg/kgにより治癒率は50%と低く，25mg/kgに増量しても73%程度であり，他の地域と比べて高用量でもメフロキン単独での治療が難しいことが示された（Smithuis *et al.*, 1993)。タイ・ミャンマーの国境付近で15mg/kgと25mg/kgとの2段階用量による検討を行ったところ，第28日での判定にて治癒率が前者では60%，後者では91%であり，タイ・カンボジア国境地帯よりは治療効果がよかった（ter

Table 1 Recent reports on the effectiveness of mefloquine therapy against falciparum malaria

Country	Age	Dose	Observation period (days)	No. of cases	Outcome (%)		Reference
					S[a]	R[b]	
Senegal	1-40	12.5mg/kg	7，14	31	93.5	6.4 (RI, 0; RII, 3.2; RIII, 3.2)	Hatin *et al.*, 1992
Nigeria	6.0±2.9[c]	15mg/kg	28	40	100	0	Sowunmi *et al.*, 1992
	5.4±2.9	25mg/kg	28	45	100	0	
Thailand	24±7.6	1,250mg	28	37	81.1	18.9	Looareesuwan *et al.*, 1992
Thai-Burmese border	All	15mg/kg	28	90	60.0	40.0	ter Kuile *et al.*, 1992
		25mg/kg	28	92	91.3	8.7	
Thai-Cambodian border	All	15mg/kg	42	58	50.0	50.0	Smithuis *et al.*, 1993
		25mg/kg	42	59	72.9	27.1	
Denmark	ND[d]	1,500mg	ND	81	98.8	1.2(RI)	Lariam® Product Monograph, 1992

[a]S: sensitive, [b]R: resistant, [c]mean±SD, [d]ND: not described

Kuile *et al.*, 1992)。

タイでは1984年からメフロキン250mg, スルファドキシン500mg, ピリメサミン25mgを含有するFansimef®(Roche)が頻繁に使われた。これはザンビア, ブラジル, ミャンマー, 中国などで1-2錠の服用にて96-100%の良好な治癒率を示した。しかし, タイで1錠服用した成績ではRIが37%にも上り, 3錠に増量して初めて98%の効果が得られたことから, 3種の薬剤を含んだ合剤としては期待した程ではなかった。その後, WHO (1990) はFansimef®をもはや推奨しないことになるが, その理由は, 1)ファンシダールの場合と同様に, 重篤な皮膚・粘膜の副作用がある。2)メフロキン単独の場合と治療効果は変わらない, 3)タイでの結果からはメフロキン耐性の出現を抑制していない, などである。

メフロキン低感受性のマラリアが多い地域では, 併用薬剤としての価値が認められている。例えば, タイにおいてメフロキン計1,250mgによる治癒率が81%, チンハオス製剤であるアーテスネート計600mg単独による治癒率が88%である状況で, 始めにアーテスネート, その後メフロキンをいずれも同量併用することで治癒率は100%に達し (Looareesuwan *et al.*, 1992), メフロキン計1,250mgとドキシサイクリン200mg/日を7日間の併用でも96%の治癒率が得られている (Looareesuwan *et al.*, 1994)。ベトナムではチンハオス製剤であるアーテミシニンの使用経験が豊富である。当初, 500mg/日で5日間の内服が行われ, 解熱, 原虫消失などの面で他の薬剤より優れていたが, 半減期が短いためかRI型再燃も多いことが難点であった。しかし最近では, アーテミシニン500mgとメフロキン500mgとの同時単回投与 (あるいは, 後者を6時間後に投与) を行っており, 28日間の観察にて外来成人患者62人(あるいは68人)の全てが再燃もなく治癒している (Hien *et al.*, 1994)。対照として, アーテミシニン500mgと6時間後にファンシダール3錠を投与する併用療法では, 46.1%が再燃を生じた。アーテミシニンとメフロキンの両薬剤の量が少ない投与法であることから, 副作用軽減の面では期待が持てる。

メフロキンは本来, 熱帯熱マラリアの治療薬として開発されたものであるが, 三日熱マラリアでも効果は示されており, タイ中央部では1,500mgの投与にて15例中全例が治癒した (Dixon *et al.*, 1985)。Semi-immuneの患者では少量でも効くことがあり, 僅か250mgの投与にて20例中18例が治癒した (Harinasuta *et al.*, 1985)。パプアニューギニアやインドネシアで報告されているクロロキン耐性三日熱マラリアが今後深刻な問題となった場合には, メフロキンによる治療が期待される。他のマラリアに対する効果については特にまとまった報告がないが, 有効であると思われる。

6. 副作用

発売前に臨床治験として行われた27件合計1,217例の集計をRoche社が行ったが, 最も多いのが眩暈で約18%, 以下悪心, 嘔吐, 下痢, 頭痛, 洞性徐脈, 胃痛, 皮膚症状, 食欲不振などが見られている (Lariam® Product Monograph, 1992)。発売後の報告での副作用を表2に示すが, それぞれの症状の出現率については様々の数値が出されている。例えば, 悪心については0-20.9%, 嘔吐については0-23.9%などと一定していない。これについては, 胃薬を併用しているか否かの違いから様々な頻度となっている可能性もある。

眩暈はメフロキンに特徴的な副作用として挙げられており, WHO (1994) もメフロキン予防投与の禁忌として, 飛行機操縦士や高所作業者などの平衡感覚を必要とする人を挙げている。しかし, 眩暈の出現率も0-79.4%とさまざまな数値が報告されている(表2)。多分に主観的である眩暈については, どの程度から有意ととるかについての違いが問題となろう。マラリアによる発熱でも眩暈は生ずるし, 事前に眩暈の副作用を伝えておくと患者が訴える率も高まる事実もある。そのような問題から, 平衡感覚機能を客観的に評価する方法が期待される。最近, 飛行機操縦士の訓練に用いるシミュレーションを利用し, メフロキンの予防投与状態で協調運動を要する操縦操作, 神経行動学的評価, 姿勢の揺れの測定などが検討されたが, 結論として, それらの項目には影響が見られなかった (Schlagenhauf,

Table 2 Frequency of adverse reactions after the therapeutic use of mefloquine

Country	Age	Dose	No. of cases	Adverse reaction (%)							Reference
				Nausea	Vomiting	Diarrhea	Abdominal pain	Dizziness	Headache	Others	
Senegal	1-40	12.5mg/kg	47	0	14.9	0	0	0	0	0	Hatin *et al.*, 1992
Nigeria	6.0±2.9[a]	15mg/kg	40	0	5.0	0	7.5	0	0	0	Sowunmi, *et al.*, 1992
	5.4±2.9	25mg/kg	45	0	15.6	2.2	13.3	0	0	0	
Thailand	24±7.6	1,250mg	43	20.9	16.3	2.3	2.3	18.6	39.5	0	Looareesuwan *et al.*, 1992
Thai-Cambodian border	32 ±11.3	<40kg, 15mg/kg; >40kg, 750mg	113	0	23.9	25.2	0	79.4	0	0	Fontanet *et al.*, 1993
Denmark	ND[b]	ND	61	0	0	0	0	55.7	14.8	24.6	Rønn *et al.*, 1995

[a]mean±SD, [b]ND: not described

Table 3 Reports on severe neuropsychiatic adverse reactions after the therapeutic use of mefloquine (modified from Weinke *et al.*, 1991)

Patient	Age/ Sex	Dose (mg)	Interval (days)	Duration (days)	Symptom
1	26/F	1,500	2	7	*Psychiatric*: acute psychosis with delusion, hyperactivity, major disturbance of sleep-wake-rhythm
2	39/M	1,500	2, 5	6	*Psychiatric*: agitation, confusion, disturbance of sleep-wake-rhythm
3	38/F	1,500	2	7	*Psychiatric*: hallucinations, confusion, disturbed consciousness, blocked thought process
4	44/F	1,500	2	5	*Psychiatric*: acute psychosis with hallucinations and paranoia, anxiety; *Seizures with convulsions*
5	26/M	1,500	1	5	*Psychiatric*: anxiety, depression, vertigo, nausea; *Neurologic*: abnormal neurological coordination, percepton disorder
6	23/F	1,500	1	5	*Neurologic*: abnormal neurological coordination, vertigo, nausea
7	42/F	1,500	1, 5	7	*Seizures with convulsions*; *Psychiatric*: confusion, blurred vision
8	49/M	1,500	2	5	*Seizures with convulsions*: leading to a fracture of the base of the skull

1995)。しかし，健常人でなくマラリア患者がメフロキンを服用した時の影響については，否定されるものではない。

重篤な副作用としては精神神経症状が挙げられ，治療の場合の出現頻度として215例中1例程度と報告されている(Weinke *et al.*, 1991)。それは精神症状，神経症状，痙攣の3種に分類され，前2者についてはさらに様々の症状が含まれる(表3)。投与1-2日後に出現することが殆どであり，中には一度改善してさらに数日後に再出現する例もある。持続期間は5-7日であり，重篤なため殆どの例で抗精神薬，抗痙攣薬を必要とした。幸いなことにそれらでの死亡例はない。全マラリア患者，メフロキン服用者は男性の方が多いと言われるが，8例中5例が女性であった。しかし，女性に出現しやすいのかどうかの結論は出ていない。用量は全て1,500mgであったが，低用量（15mg/kg，成人で750-1,000mg)で治療した場合にも出現するのかは不明である。多くの例ではクロロキン，キニーネなどの他の抗マラリア薬を，それ以前にあるいは同時に服用していたが，それらが真の危険因子であるのかについても不明である。

7．当院における使用経験

厚生省『熱帯病治療薬の研究開発班』（班長：慈恵医大・大友弘士教授）が対象薬剤の一つとしてLariam®を輸入し，国立衛生試験所での検定を経て，全国13ケ所の熱帯病を扱う医療機関に配付していることから，当院でも1992年6月頃より熱帯熱マラリア患者に使用する機会が増えた。今回，retrospectiveではあるがそれらの集計・解析を行い，日本におけるマラリア治療を考える上での参考に供したい。メフロキン単独で治療を開始したのは28例であったが，このうちの1例は服用後直ちに嘔吐したため，メフロキンによる治療を断念して他の治療に切り替えた。従って，メフロキン単独治療例としては27例，副作用を集計する際のメフロキン投与例としては28例である。

感染地域については熱帯アフリカが20例で最も多く，その他大洋州，東南アジアであった。年令は8-48歳に分布し，平均34歳であり，男女比は22：5であった。外国人が7例含まれていた。治療前の原虫数は151-66,420/μl，平均12,021/μlであった。投与量は成人26例では750～1,500mgであり，8歳の小児1例では500mgであった。成人投与量については初期の頃，Roche社の説明書に従い，non-immuneでは体重≥60kgで1,500mg，<60kgで1,250mgとしていたが，1993年4月からは殆どのnon-immuneの症例でも，semi-immuneの投与量として示されている体重≥60kgで1,000mg，<60kgで750mgによる治療とした。また同じ頃から，嘔吐を防ぐために胃薬（マーズレンS 2.0/日，ストロカイン3-6錠/日の併用）を同時に，あるいは事前から投与している。

27例中1例を除いた26例では，治療開始後7日以内に原虫の消失が見られ，最短でも4週間の観察を行った結果では再燃が見られず，WHOの判定基準で“S”と判定された。これらの例について経過を詳細に見ると，治療開始1日後には原虫数が減少する例のみならず，増加する例もしばしば見られたが，いずれも2日後の検査では減少傾向が見られた。原虫の膨化，萎縮，赤血球内の斑点の出現などの形態変化は治療開始1日後に現れ，原虫数の減少と共により顕著になった。原虫数が1日後に増加する例でも，同様な変化は同日に見られた。発熱消失日数は2.9±1.2(SD)日であり，原虫消失日数は2.7±1.0日であった。メフロキンにより治癒した1例の経過を図2に示す。

メフロキン治療に反応が見られなかった1例はギニア共

Laboratory data

Hb	16.2	16.1	14.0	15.5	13.8	13.6	13.9	12.6
WBC	5,170	3,510	3,460	5,640	6,200	6,800	6,700	6,710
Plt	9.9	5.2	2.5	2.2	3.0	7.0	9.8	15.3
GOT	201		111		70			69
GPT	158		168		115			98
LDH	792		738		544			440
T-Bili	1.0		2.8		2.8		1.1	0.9
BUN	9.4		25.7		25.0		15.6	11.0
CR	1.1		1.3		1.4		1.1	1.0

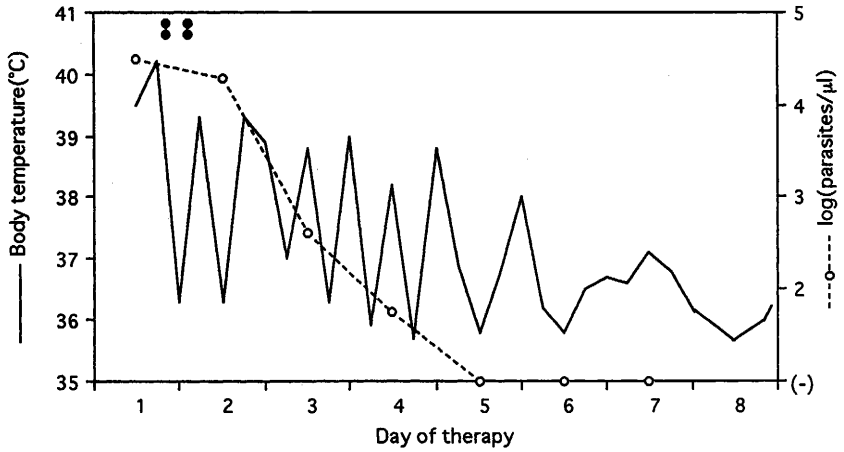


Figure 2 Falciparum malaria of a 48-year-old male acquired in Laos which was successfully treated with mefloquine. With a total of 4 tablets Lariam® (●: 250mg of mefloquine base), his fever subsided 7 days and the parasites became undetectable 4 days after the start of therapy. Decreased platelet count, elevated values of transaminases, lactate dehydrogenase, bililubin, blood urea nitrogen and serum creatinine were almost or completely normalized at the end of the hospitalization.

和国での感染例であり，体重が53kgであったため計750mgを投与した。治療開始1日後の段階では，原虫数は減少しなかったが形態の変化が顕著であったため，治療に反応する通常のパターンと思われた。しかし，2日後にも原虫数の減少は見られず，それまで変化していた原虫の形態が正常の状態に戻ったため，RIII耐性と判断して（神田，1992），キニーネ点滴静注とテトラサイクリン経口との併用療法に切り替え，その後は順調に原虫が消失し再燃も見られていない。

逆に，パプアニューギニアで感染した1例は，初回ハロファントリン計1,500mgの標準的投与で原虫の消失が見られたが，その後約1週間で再燃したためメフロキン1,000mgを用い，それにより治癒した。試験管内では，メフロキン耐性とハロファントリンあるいはキニーネ耐性との相関が報告されてはいるが（Mockenhaupt, 1995），今のところ臨床的には問題となることは多くないと思われる。

副作用については，検査上での検討が充分な例は多くなく，主に自覚症状での検討となった。服用した28例のうち，何らかの副作用が認められた例は10例で35.7%であった。それらの副作用を表4に示すが，殆どは軽度でそのための治療を必要とせずに自然軽快した。落ち着きのなさ，息苦しさなどを訴えた1例では，他覚的に異常所見はなかったが念のため酸素吸入などの治療を行い，約1日の経過で後遺症もなく軽快した。

Table 4 Adverse reactions observed in 28 mefloquine-treated patients in our hospital

Gastric symptoms (nausea, vomiting, discomfort)	6 (21.4%)
Diarrhea	3 (10.7%)
Dizziness	3 (10.7%)
Finger tremor	1 (3.6%)
Restlessness	1 (3.6%)

(Institute of Medical Science, Univ. Tokyo)

現在のところ，日本人での合併症のない熱帯熱マラリアにおいて，メフロキンを第一選択薬剤としてよいものと考えている。

【謝辞】Lariam®を供与して頂いた厚生省『熱帯病治療薬の研究開発班』（班長：慈恵医大・大友弘士教授）に深謝する。

【文　献】

1) Dixon, K.E., Pitaktong, U. and Phintuyothin, P. (1985): A clinical trial of mefloquine in the treatment of *Plasmodium vivax* malaria, Am. J. Trop. Med. Hyg., 34, 435-437

2) Edstein, M.D., Veenendaal, J.R. and Hyslop, R. (1988): Excretion of mefloquine in human breast milk, Chemotherapy, 34, 165-169

3) Fontanet, A.L., Johnston, B.D., Walker, A.M., Rooney, W., Thimasarn, K., Sturchler, D., Macdonald, M., Hours, M. and Wirth, D.F. (1993): High prevalence of mefloquine-resistant falciparum malaria in eastern Thailand, Bull. W.H.O., 71, 377-383

4) Harinasuta, T., Bunnag, D., Lasserre, R., Leimer, R., Vinijanont, S. (1985): Trials of mefloquine in vivax and of mefloquine plus 'Fansidar' in falciparum malaria, Lancet, i, 885-888

5) Hatin, I., Trape, J.-F., Legros, F., Bauchet, J. and Le Bras, J. (1992): Susceptibility of *Plasmodium falciparum* strains to mefloquine in an urban area in Senegal, Bull. W.H.O., 70, 363-367

6) Hien, T.T., Arnold, K., Hung, N.T., Loc, P.P., Dung, N. T., Cuong, B.M., Toan, L.M., Phung, M.Q., Anh, L.H.V. and Mai, P.P. (1994): Single dose artemisinin-mefloquine treatment for acute uncomplicated falciparum malaria, Trans. R. Soc. Trop. Med. Hyg., 88, 688-691

7) 神田錬蔵 (1992)：多剤耐性マラリアの治療と予防，熱帯，25，1-13

8) Lariam® Product Monograph (1992), F. Hoffmann-La Roche Ltd, Basel

9) Looareesuwan, S., Viravan, C., Vanijanonta, S., Wilairatana, P, Charoenlarp, P., Canfield, C.J. and Kyle, D.E. (1994): Randomized trial of mefloquine-doxycycline, and artesunate-doxycycline for treatment of acute uncomplicated falciparum malaria, Am. J. Trop. Med. Hyg., 50, 784-789

10) Looareesuwan, S., Viravan, C., Vanijanonta, S., Wilairatana, P, Suntharasamai, P., Charoenlarp, P., Arnold, K., Kyle, D., Canfield, C. and Webster, K. (1992): Randomized trial of artesunate and mefloquine alone and in sequence for acute uncomplicated falciparum malaria, Lancet, 339, 821-824

11) Mockenhaupt, F.P. (1995): Mefloquine resistance in *Plasmodium falciparum*, Parasitol. Today, 11, 248-253

12) Na Bangchang, K., Davis, T.M.E., Looareesuwan, S., White, N.J., Bunnag, D. and Karbwang, J. (1994): Mefloquine pharmacokinetics in pregnant women with acute falciparum malaria, Trans. R. Soc. Trop. Med. Hyg., 88, 321-323

13) Oduola, A.M.J., Sowunmi, A., Milhous, W.K., Kyle, D.E., Martin, R.K., Walker, O. and Salako, L.A. (1992): Innate resistance to new antimalarial drugs in *Plasmodium falciparum* from Nigeria, Trans. R. Soc. Trop. Med. Hyg., 86, 123-126

14) Palmer, K.J., Holliday, S.M. and Brogden, R.N. (1993): Mefloquine. A review of its antimalarial activity, pharmacokinetic properties and therapeutic efficacy, Drugs, 45, 430-475

15) Rønn, A.M., Rønne-Rasmussen, J.O. and Bygbjerg, I.C. (1995): Delay in discovering significant side effects of antimalarials: the example of mefloquine, Abstracts of the 4th International Conference on Travel Medicine, p110

16) Schlagenhauf, P. (1995): Effect of mefloquine 'prophylaxis' on performance in Swissair trainee pilots. Fourth International Conference on Travel Medicine, In 'Spotlight supplement 95-4', p5, Roche, Basel

17) Slutsker, L.M., Khoromana, C.O., Payne, D., Allen, C.R., Wirima, J.J., Heymann, D.L., Patchen, L. and Steketee, R.W. (1990): Mefloquine therapy for *Plasmodium falciparum* malaria in children under 5 years of age in Malawi: *in vivo/in vitro* efficacy and correlation of drug concentration with parasitological outcome, Bull. W.H.O., 68, 53-59

18) Smithuis, F.M., van Woensel, J.B.M., Nordlander, E., Wanta Sok Vantha and ter Kuile, F.O. (1993): Comparison of two mefloquine regimens for treatment of *Plasmodium falciparum* malaria on the northeastern Thai-Cambodian border, Antimicrob. Agents Chemother., 37, 1977-1981

19) Sowunmi, A., Oduola, A.M.J., Salako, L.A., Ogundahunsi, O.A.T., Laoye, O.J. and Walker, O. (1992): The relationship between the response of *Plasmodium falciparum* malaria to mefloquine in African children and its sensitivity *in vitro*, Trans. R. Soc. Trop. Med. Hyg., 86, 368-371

20) ter Kuile, F.O., Nosten, F., Thieren, M., Luxemburger, C., Edstein, M.D., Chongsuphajaisiddhi, T., Phaipun, L., Webster, H.K. and White, N.J. (1992): High-dose mefloquine in the treatment of multidrug-resistant falciparum malaria, J. Infect. Dis., 166, 1393-1400

21) Weinke, T., Trautmann, M., Held, T., Weber, G., Eichenlaub, D., Fleischer, K., Kern, W. and Pohle, H.D.(1991): Neuropsychiatric side effects after the use of mefloquine, Am. J. Trop. Med. Hyg., 45, 86-91

22) W.H.O. (1990): Practical chemotherapy of malaria: Report of a WHO Scientific Group, WHO Technical Report Series, No. 805

23) WHO (1994): Malaria. In "International Travel and Health", p65-79, World Health Organization, Geneva

Review

CURRENT STATUS OF ANTIMALARIAL THERAPY WITH MEFLOQUINE

MIKIO KIMURA

Received November 30, 1995/Accepted January 11, 1996

Mefloquine is a quinoline compound closely related to quinine and is characterized by its long half-life and excellent clinical efficacy against chloroquine-resistant falciparum malaria. Even recently, the cure rate for falciparum malaria after its use is over 90% in many parts of the world such as most of African countries. However, it is reported to be less effective in Thai-Cambodian or Thai-Burmese border. The higher dose of mefloquine, e.g. 25mg/kg, is required in these areas, but even so unsatisfactory results often ensue. In these areas, mefloquine is used combined with other antimalarials such as qinghaosu derivatives or tetracyclines showing better results. Reported adverse reactions after the therapeutic use of mefloquine include dizziness, nausea, vomiting, diarrhea, headache and so on. With regards to dizziness, however, various figures of its occurrence are reported, probably due to its subjective nature. In addition to the above symptoms, we have to be extemely cautious about the severe neuropsychiatric reactions caused by mefloquine that are reported to occur in one out of 215 cases.

We analyzed the data on 27-28 patients with falciparum malaria who were treated in our hospital with 750-1,500mg of mefloquine. All but one case were successfully treated without later recrudescence. About one third of the cases experienced undesirable symptoms attributable to the medication, however, most of them were slight and did not require medical intervention. It is likely that mefloquine could be a first-choice drug for uncomplicated falciparum malaria in Japanese as well as in other people.

Department of Infectious Diseases and Applied Immunology, Institute of Medical Science, University of Tokyo

Jpn. J. Trop. Med. Hyg., Vol. 24, No. 1, 1996, pp. 35-82

PROCEEDINGS OF XXXVII ANNUAL MEETING OF JAPANESE SOCIETY OF TROPICAL MEDICINE

29 November-1 December 1995, Nagoya

President

Shin Isomura

Professor=Department of Medical Zoology,
Nagoya University, School of Medicine

CONTENTS

A—39 Drancunculiasis (guniea worm disease) M. Aikawa *et al.*
A—40 Study on schistosoma sinensium in northwest Thailand M. Kawanaka *et al.*
A—41 Presence of *Schistosoma haematobium* eggs in venous blood examined for *Wuchereria bancrofti* microfilariae Y. Fujimaki *et al.*
A—42 An imported case of a Japanese patient infected with *schistosoma mansoni* K. Ohnishi *et al.*
A—43 Ultrasonographic study of liver fibrosis in the patients with schistosome esophageal varices in northeast Barzil M. Tanabe *et al.*
A—44 A case of a 1-year-old infant suspected of cysticercosis with multiple space occupying lesions in brainstem K. Hibino *et al.*
A—45 Present state of serodiagnosis in echinococcoi and cysticercosis A. Ito
A—46 Therapeutic effects of combination of diethylcarbamazine and sodium bicarbonate on bancroftian filariasis Y. Fujimaki *et al.*
A—47 Preparation for vesicles of *echinococcus multilocularis* and colorimetric quantitation of the viability of germinal cells T. Kanazawa *et al.*
A—48 *Angiostrongylus cantonensis* infection in IL-5 transgenic mice with special reference to their resistance to infection and adhesion molecule expression in eosinophils H. Sugaya *et al.*
A—49 Eosinophil-specific expression of gp91-*phox* A. Kumatori *et al.*
A—50 Pcr primer to detect genomic DNA of *Toxocara canis* and *T. cati* Y. Takahashi *et al.*
A—51 Characterization of a gene for the 34kDa eggshell precursor protein of *Schistosoma japonicum* H. Sugiyama *et al.*
A—52 Mapping of a newly isolated eggshell precursor protein gene on *schistosoma japonicum* chromosomes Y. Kameoka *et al.*
A—53 A molecular phylogeny of the genus *Paragonimus* from a viewpoint of ITS 2 region of the nuclear ribosomal RNA genes T. Agatsuma *et al.*
A—54 Diagnosis of diarrheal cases in Rwandan refugees in Goma, Zaire H. Shiomi *et al.*
A—55 Immunological background in infants with diarrhea in Ghana K. Taniguchi *et al.*
A—56 Structure and function of recombinant pig receptor (STaR) for a *Escherichia coli* heat-stable enterotoxin (STa) Ⅳ: characterization of carboxy-terminal region of heat-stable enterotoxin receptor A. wada *et al.*
A—57 *Epidemiology of influenza virus infections among children with acute respiratory infections (ARI) in Zambia.* K. Mizuta *et al.*
A—58 Utility of Polymerase Chain Reaction (PCR) Method for Rapid Laboratory Diagnosis of Enterovirus Infection T. N. Dohle *et al.*
A—59 Characterization of polioviruses Isorated from West Pakistani children A. Hasegawa *et al.*
A—60 Measles infection in HIV seropositive children in Zambia H. Oshitani *et al.*
A—61 Comparative analysis of anti-tuberculous chemotherapy between 2REHZ/6RH and 2SEH/10EH for HIV-seropositive patients with pulmonary tuberculosis in Uganda S. Mitarai *et al.*
A—62 An epidemiological study on kaposi's sarcoma in western kenya for 15 years K. Toriyama *et al.*
A—63 Virological study on an imported dengue fever case K. Yamada *et al.*
A—64 Seroepidemiological and virological studies on Japanese encephalitis in Vientiane, Lao P. D. R. M. Saito *et al.*
A—65 Inhibitory effect of suramin on Japanese encephalitis virus replication K. S. Xu *et al.*

Prize Winner's Lecture

DNA DIAGNOSIS OF MALARIA

MEIJI ARAI
Department of Internal Medicine, Okayama Kyoritsu General Hospital

We have developed two DNA diagnostic methods using polymerase chain reaction (PCR). Double PCR is a method for detection of *Plasmodium falciparum* parasites in infected human blood using two-step PCR. *P. falciparum*-specific sequence of the DHFR-TS junction region was amplified with two sets of oligonucleotide primers and the PCR-product was analyzed by agarose gel electrophoresis. In 1991, 101 samples from 98 donors were collected in Guadalcanal, Solomon Islands, and subjected to the double PCR assay. We encountered a case of falciparum malaria, whose clinical symptoms and the results of the double PCR had changed in parallel with the drug treatment.

Microtiter plate-hybridization (MPH) is a colorimetric assay for detecting and differentiating the four species of human malaria parasites, *P. falciparum* (PF), *P. vivax* (PV), *P. ovale* (PO), and *P. malariae* (PM). A pair of oligonucleotide primers were designed for the amplification of the conserved region of the gene encoding for the 18S rRNA. Species-specific oligonucleotide probes were also designed and immobilized on microtiter wells. Target sequence of malaria parasites in human blood was amplified and the PCR-product was captured by the species-specific probe on the microtiter well. The biotin-streptavidin system was used to detect the captured materials.

PF-specific and PV-specific probes of MPH were tested in Guadalcanal in 1993. Among the 130 blood samples, 30 (23%) were positive by PF-specific probe, 28 (22%) were positive by PV-specific probe, and 8 (6%) were positive by both of them. These results of MPH were similar to those of acridine orange microscopy.

We evaluated MPH by using blood specimens from malaria patients. Among 504 blood samples tested, all of 44 cases of falciparum malaria, 27 of 28 cases of vivax malaria, 12 of 13 cases of ovale malaria, and 2 cases of malariae malaria were diagnosed species specifically by MPH. In this study, the restults of MPH showed close correlation with the clinical course of each case.

MPH was also evaluated in Viet-Nam, in 1994 and 1995. We encountered a case of microscopically PO-positive but MPH-negative. Sequence analysis of the PCR-product of this case revealed that the target sequence for the PO-specific probe was different from the published sequence of PO, suggesting that there may be two kinds of PO parasites.

MPH will be valuable for large-scale epidemiological studies, analysis of new species or variant types of malaria parasites, management of malaria in clinical practice, especially in early diagnosis, species differentiation, and assessment of treatment.

Symposium

MEASLES AND MEASLES VACCINE IN DEVELOPING COUNTRIES

SHIN ISOMURA
Department of Medical Zoology, Nagoya University School of Medicine

Despite the activities of the Expanded Programme on Immunization (EPI) which have resulted in a dramatic decrease of mortality and morbidity of measles, the disease still remains one of the killer diseases in children; in 1990 45 million cases and around 1 million deaths were estimated in developing countries. This report introduces some epidemiological findings on measles and measles vaccine obtained through field surveys in Ghana, West Africa and Karachi, Pakistan conducted in 1982-1994.

(1) High morbidity and high case fatality rate during infancy have been one of the characteristics of measles in developing countries. The morbidity and mortality have been closely related to underling malnutrition, and other serious complications have so often developed after the contraction of measles.

(2) Loss of potency of measles vaccine because of insufficient cold chain network has been main reason of low efficacy of the vaccine administration.

(3) To increase the coverage of immunization, community-based surveillance on the information resources to mobilize mothers to participate EPI. Grass loot activities for maternal child health care including literacy school should be promoted in each community.

S—1

HIV INFECTION AND AIDS IN VIETNAM AND MYANMAR

SHUDO YAMAZAKI, AND YUTAKA TAKEBE
National Institute of Health

In March 1995, we conducted on-the-spot surveys for HIV-infected cases in Myanmar and Vietnam, in both of which among Asian countries HIV infection has markedly increased recently, to realize the present status of HIV infection and to promote an international cooperative investigation to contribute preventive measures against AIDS.

Invasion of AIDS to Vietnam and Myanmar, situated respectively at the east and west ends of the Indochina Peninsula was found 10 years later than that to American and European countries, and the reports of HIV-infected cases have rapidly increased since 1990 (Table 1). It has been pointed out that the epidemiological features common to both countries are that the main route of transmission used to be via blood by exposure through needle-sharing among intravenous-drug users (IVDU), followed by rapidly increased sexually transmitted disease (STD) cases among commercial sex workers (CSW) and, more recently, a tendency of rapid spreading of infection among such high-risk groups as IVDU and CSW to ordinary populations. However, HIV infection can still be found frequently among IVDU. For instance, a dreadful report tells that more than 90% of IVDU are infected with HIV in Mitkyina in Kachin State, situated in north Myanmar adjoining to China. HIV-infected cases have increased year by year since 1992 among CSW concentraed in such urban districts as Yangon and Mandalay. It is particularly noticeable that infected cases increased suddenly to four times as many in Mandalay during the two years from 1992 to 1994.

To cope with such serious HIV/AIDS epidemics, the Myanmar Ministry of Health, according to the instruction given by the National Health Committee,

Table 1 HIV Infections

	Myanmar	*Vietnam*
1988	1	
1989	323	
1990	1034	1
1991	2152	0
1992	1641	11
1993	2001	1124
1994	1039(-June)	1148
Total	8191	2284

has established sentinel stations in the whole country and been conducting surveillance in 19 cities since March 1992. In Vietnam, the National AIDS Committee has been organized since 1989 and a monthly committee meeting is being held to discuss on countermeasures against AIDS on the basis of the reports from the HIV surveillance operation group. In 1992, a nation-wide surveillance system was established. By order of the Ministry of Health, the National Institute of Hygiene and Epidemiology (NIHE) in Hanoi in the north and Pasteur Institute in Ho Chi Minh in the south are striving to strengthen and enrich the functions as the National Reference Centers to perform confirmatory tests on HIV-infected cases and analyze the epidemiological data on patients and infected cases. Giving economical, technical and manpower assistance to these countries by Japan and other developed countries is being strongly desired.

S—2

CURRENT SITUATION OF HIV EPIDEMIC IN THE KINGDOM OF CAMBODIA

MASAHIRO KIHARA
Department of Epidemiology, Kanagawa Cancer Center Res. Inst

Combodia, with a population of about 9 million, has been on a difficult way of reconstruction after the prolonged and devasting civil war. However, it has become increasing clear that this country has had to face another challenge, HIV epidemic.

Since the first HIV positive (HIV+) case was identified in 1991, 13 AIDS case and 1225 HIV+ were already reported as of April in 1995. By transmission mode, majority of them were of heterosexual contact or unknown transmission category with no or little cases of homosexual contact and intravenous drug use (IDU). Sentinel surveillance started 1994 has revealed that HIV infection has been spreading with an unprecedented rate in this country, already reaching the level which is equivalent or even worse than that in Thailand. HIV infection rate was 4.3% among blood donors in 1994, a 50-fold increase from 0.08% in 1991, 9.2% among commercial sex workers (CSW) in Phnom Penh in 1992, 38. 2% among CSW in Sihanoukville in 1994 and 9.2% STD patients in Phnom Penh in 1994.

Growing population of CSW, estimated now to be 2, 000 in Phnom Penh and 10,000 countrywide is one of the greatest contributing factors to the epidemic, which started to grow during the period of United Nations Peace-Keeping Forces (UNTAC) and stimulated by the following economic liberation. Preference of Cambodian people for injection as a mode of treatment of illuness is another potential factor for epidemic because of a very limited availability of disposable needles or syringes and equipment for sterilization. Limited opportunity of HIV testing also provides the risk through transfusing unscreened blood especially in peripheral regions and economical growth and associated urban migration have been increasing the threat for futere increase in IDU.

Importance of urgent international aids for HIV/AIDS control program of this couontry cannot be overstressed.

S—3

CURRENT EPIDEMICS OF HIV AND AIDS IN THE TROPICAL ASIA: THAILAND

KAZUO TAJIMA
Division of Epidemiology, Aichi Cancer Center Research Institute

The first case of AIDS in Thailand was reported in 1984. This was followed by an explosive spread of HIV infection among injecting drug users and then spread to male and female commercial sex workers CSWs and their clients by heterosexual transmission. In 1987 the first case of HIV infection by blood transfusion was reported and since 1989 Thailand initiated to screen every unit of donated bloods for anti-HIV antibody.

Since 1991 the Thai government has relied on the AIDS Voluntary Reporting System, however, under reporting of AIDS cases remains problematic as in most countries. By the November 1995 the number of total cases of AIDS was counted to 22,135. Among then 75% and 8% were caused by sexual transmission and injecting drug use, respectively. The proportion of vertical transmission from mother to child was about 8%. At present the cumulative number of HIV carriers in Thailand was estimated at 700,000 and the government projected 2-3 million HIV infections by the year 2000. Health education is the most important component in the Thailand's AIDS prevention program and they are broadcasting correct information on HIV prevention measures through TV everyday. And furthermore, since 1990, the effective 100% condom usage program was promoted and the owners of sex establishments were educated that all CSWs will not be able to purchase sex services without condom use in any sex establishment. It was reported in 1992 that more than 90% of CSWs took condom, and since 1990 the prevalence of the sex transmitted diseases were actually decreasing. The two critical issues on AIDS in Thailand are : 1) how to establish the future care system for lots of AIDS patients progressed from the huge amount of HIV carriers; and 2) how to control the HIV spreading route from injective drug users to general people.

S—4

HIV/AIDS IN INDIA AND INDONESIA

TAKESHI KURIMURA
Department of Viral Infections, Research Institute for Microbial Diseases, Osaka University

Among Asian countries, India and Indonesia have very characteristic features. Both countries have large population size of 870M and 180M, respectively. India shares its border with other countries but Indonesia is a typical island country consisted of many islands.

[India] India is expected to have the biggest number of HIV carriers in the world but the surveillance system is not working well. At the moment, 2.5M to 3.0M people are infected with HIV type 1, type 2 or both types. National Commission on AIDS started in August 1995 to implement AIDS control strategies in India. Several Indian excellent research groups reported the situation of HIV/AIDS in Bombay, Maharashutra. Maniar's group first reported the existence of HIV type 2 as well as type 1 in Bombay and Hira's group reported dual infections with both types are as frequent as 20% of the total HIV carriers. This is quite in good contrast with the data by Kanki and Essex on the apparent protective ability of prior HIV type 2 infection against HIV type 1. A large number of female CSW's exist in India and high prevalence HIV infections among them has been reported together with high prevalence of tuberculosis. Gilada's group, Bhave's group and others are working on the HIV/AIDS problem, STD's and tuberculosis of CSW's in collaboration with other NGO's. Poverty, low literacy rate etc. are the obstacles against STD/AIDS control. Well-organised AIDS control programs such as extensive safe blood policy, education, distribution of condoms are very important.

[Indonesia] Since the announcement of the Presidential Decree Nr. 41 in April, 1994, systematic control policy against AIDS has been started. By the end of October, 1995, 355 HIV-infected cases have been reported and majority of them were in Jakarta and Bali. 120 of these 355 were foreigners. The most probable route of HIV transmission is sexual contact, male-to male and male to female. In Irian Jaya, many Thai fishermen were found to be seropositive (50 of total number 64). Male to female ratio of HIV infection was 3.1: 1 by the end of October, 1995, while it was 3.4: 1 by the end of November, 1994. This may reflect the increase of HIV incidence of women. Indonesia has 5 major religion groups, Muslim, Hindu, Buddhism, Catholic and Protestant and the government is distributing 5 different pamphlets for AIDS education, Although the number of HIV infections is small, many NGO's are actively working on the control of HIV/AIDS. The high prevalence of STD's will promote the spread of HIV and they have been amply introduced in Indonesian population. Education and STD control are the most important for Indonesia to keep the low prevalence of HIV/AIDS.

General Presentation

A—1

CORRELATION BETWEEN DNA POLYMERASE ACTIVITY AND VIRULENCE OF *TOXOPLASMA GONDII*

ASAO MAKIOKA AND HIROSHI OHTOMO
Department of Tropical Medicine, Jikei University School of Medicine

We have previously detected and characterized DNA polymerase activity of the virulent RH strain of *Toxoplasma gondii*. There is variation in virulence from strain to strain of *T. gondii*. Although the reason for this strain difference remains unclear, it is considered that an increased rate of proliferation of tachyzoites is closely correlated with faster destruction of the infected hosts cells. In the present study, we have compared DNA polymerase activity of virulent RH strain with that of avirulent ME49 strain in relation to their virulence. Marked difference in virulence between both strains was confirmed by comparing the mortality and survival time of mice infected with these strains. Experiments of *in vitro* culture system have indicated that the number of RH tachyzoites within infected macrophages was about twice as many as that of ME 49, suggesting that the rate of proliferation of RH strain is significantly faster than that of ME49 strain. DNA polymerase activity of RH strain was about 2.5 times higher than that of ME49 strain, while sensitivity of the activities of both strains to salt, Mg^{2+} and inhibitors of mammalian DNA polymerases were almost similar. Thus it is suggested that this increased activity of the enzyme of virulent strain contributes to a faster rate of multiplication of the organisms as compared with that of avirulent strain.

A—2

ANALYSIS OF POLYMORPHISM OF THE GENE ENCODING THE CYSTEINE PROTEASE FROM DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI*

MARÍA PAULA DE LEÓN[1,2], KENJI HIRAYAMA[1], VIVIAN MATTA[2], TETSUO YANAGI[2,3], MARGARITA PAZ[2] AND MIHOKO KIKUCHI[1].
[1]Department of Medical Zoology[1], Saitama Medical School, Japan; Dept. of Cytology, Faculty of Chemical Sciences and Pharmacy, USAC, Guatemala[2]; Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Japan[3]

With the aim of detecting polymorphism among guatemalan isolates of *Trypanosoma cruzi*, epimastigotes from 55 local isolates and 14 nominal laboratory strains (from Brazil, Ecuador, Chile, Colombia, Paraguay) were analyzed by amplification of the cystein protease gene and the single strand conformation polymorphism (SSCP) method. The guatemalan isolates were obtained in endemic areas of the Chagas' disease from which, 44 are from vector insect (*Triatoma dimidiata*) and 11 from infected humans.

We could obtain the PCR products from all the analyzed samples and those showed no difference in their size (approx. 450 bp). Comparison of the SSCP led to the grouping of several local isolates and nominal strains into 5 different migration patterns. The first pattern was the most common among the local isolates from vector insect and infected humans. The same pattern is shown by the Colombia and CL strains which are from Colombia and Brazil respectively. The second pattern was shown by some of the local isolates from vector insect but not from infected humans. Likewise, we found that the strain named 119 which was isolated from opossum in Ecuador shows the same pattern. An interesting finding is that just one local isolate from infected human showed a different pattern from the other local ones, even from the nominal strains, therefore was classified as pattern No. 3. The fourth pattern was found in the Y and Bernice (Brazil) strains and

Tulahuen-L (Chile), LO, GS, RF (Paraguay) strains as well, but not any local isolate from vector or infected human. The final pattern (No. 5) was observed only in the Tulahuen-H strain (Chile).

Taken together, we have identified three different types (patterns 1, 2, 3) of cystein protease gene from the local isolates and the first one was the most common, either in vector as infected humans, suggesting that type 1 was related to human infective type. The nucleotide sequencing study is now going on in our laboratory.

A—3

MOLECULAR CHARACTERIZATION OF A FRAGMENT OF *LEISHMANIA* GENE ENCODING CARBAMOYL-PHOSPHATE SYNTHETASE II (CPSII)

GUANGHAN GAO, JUNKO NAKAJIMA-SHIMADA, HIROSHIYAMASAKI,
TAKESHI NARA, AND TAKASHI AOKI
Department Parasitol, Juntendo University

Carbamoyl-phosphate synthetase II (CPS II) is a key enzyme in nucleic acid synthesis, which catalyzes the first step in de novo pyrimidine nucleotide biosynthesis. In *Escherichia coli*, CPS II is composed of a light and a heavy subunit encoded by *carA* and *carB*, respectively. The light subunit has the activity of L-glutamine amide transferase (GAT), while the heavy one utilizes the transferred amide nitrogen in the reaction of carbamoyl-phosphate synthetase (CPS). The latter reaction requires ATP. By contrast, in animals including helminth parasites and mammals, CPS II occurs as a multidomain protein (GAT-CPS-DHO-ACT) with aspartate carbamoyltransferase (ACT) and dihydroorotase (DHO), the second and third enzymes of the pyrimidine pathway.

The purpose of this study was to characterize a gene encoding *Leishmania mexicana amazonensis* (*Lma*) CPS II. A polymerase chain reaction (PCR), using *Lma* genomic DNA as template, yielded a fragment of 1164 bp. This was subcloned and determined for the nucleotide sequence. The deduced 388 amino acid residues consist of 3 parts, namely, C terminus of GAT (68 residues), a short polypeptide linker (15 residues), and N terminus of CPS (305 residues). This observation enlarges our previous finding in *T. cruzi* that a polypeptide carries GAT and CPS domains, separated from both of ACT and DHO, a new type CPS II that resembles neither of *E. coli* nor animal CPSs II. An alignment of the *Lma* 388 amino acid residues with those of various sources highlights the following characteristic sequence motifs. In the C terminus of *Lma* GAT, there are two highly conserved regions, TSQNHGFAVD (consensus: TSQNHGFAVD) occurring only in GAT of various CPSs II and PFCSVQFHPE (consensus: PXFSVQFHPE) containing histidine and glutamate residues that constitute an active site of GAT. The hydrophilic linker portion VKGSKVKEVAKFKPR shares a strikingly high homology with that from *T. cruzi* (VKESKV KEASKYKPR). In the remainder 305 residues, an ATP-binding site GGQGSGIVENMAE is identified. This 305 amino acid sequence is highly homologous to those of *E. coli* (*carB*), yeast (*CPA2* and *URA2*), rat mitochondria (CPS I), and *T. cruzi* (CPS II), entailing the calculated percentages of identical residues of 47.9, 61.8, 62.0, 61.5, and 74.7%, respectively. The result indicates that, phylogenetically, the *Lma* CPS II is more closely related with eukalyotic than prokaryotic enzymes, and is most intimately related with the *T. cruzi* enzyme.

A—4

THE P-GLYCOPROTEIN GENE FAMILY IN *LEISHMANIA*: MOLECULAR CLONING OF A NEW P-GLYCOPROTEIN-RELATED GENE FROM *LEISHMANIA AMAZONENSIS*

KEN KATAKURA[1], HIROSHI OHTOMO[1] AND HITOSHI SUZUKI[2]
Department of Tropical Medicine[1] and Division of Molecular Genetics[2], Jikei University School of Medicine

ATP-binding cassette (ABC) transporters are known to utilize the energy of ATP hydrolysis to transport substrate across the membrane and be associated with important biological processes in both prokaryotes and eukaryotes. Among over 50 ABC transporters, human P-glycoprotein (MDR1), for example, exports multiple anticancer agents across the cell membrane, enabling cancer cells multidrug resistant against such drugs. A protozoan parasite, *Leishmania*, contains several P-glycoprotein genes. Two different functional genes (*ldmdr1* and *ltpgpA*) have been isolated from different *Leishmania* species, and demonstrated to mediate resistance against specific drugs. In this study, we aimed to isolate and characterize P-glycoprotein genes in *L. amazonensis*, one of the causative agent of leishmaniasis in the New World. Polymerase chain reaction (PCR) was performed to amplify ATP binding motif of *L. amazonensis* P-glycoproteins using primers designed for conserved sequences of ATP binding motif among P-glycoprotein genes from different organisms. We detected a 408-bp PCR product which showed substantial similarity to N-terminal ATP binding motif of *ldmdr1* and *ltpgpA*. A genomic library of *L. amazonensis* DNA was screened with the PCR product and we detected a 3801-bp open reading frame from a positive clone. The putative 1267 amino acids indicated the transmembrane P-glycoprotein like structure and showed 46, 39, and 29% identity to *ldmdrl, MDR1* and *ltpgpA*, respectively. Furthermore, pulsed field gel electrophoresis and Southern blot hybridization analyses indicated that this P-glycoprotein-related gene was single copied. These results suggest that *L. amazonensis* contains at least three different P-glycoprotein genes, the *ldmdr1* homologue, *ltpgpA* homologue, and new gene isolated in this study. Functions of the gene is under investigation.

A—5

DIVERSITY IN BLOCK 4 OF MEROZOITE SURFACE PROTEIN 1 (MSP1) OF *PLASMODIUM FALCIPARUM* IN NATURAL POPULATIONS

OSAMU KANEKO[1], SOMCHAI JONGWUTIWES[2], MASATSUGU KIMURA[3]
HIROJI KANBARA[4], AKIRA ISHII[5] AND KAZUYUKI TANABE[6]
Department of Medical Zoology[1], Laboratory of Biophysics[3], Osaka City University Medical School, Japan; Department of Parasitology, Faculty of Medicine, Chulalongkorn University[2], Thailand; Department of Protozoology, Institute of Tropical Medicine, Nagasaki University[4]; Department of Medical Biology and Parasitology, Jichi Medical School[5]; and Laboratory of Biology, Osaka Institute of Technology[6], Japan

Merozoite surface protein 1 (MSP1) of *Plasmodium falciparum* is one of the potential candidates for a sexual blood-stage malaria vaccine. MSP1 consists of several conserved blocks interspersed with variable blocks. Although MSP1 exhibits antigenic diversity, variations in the gene are not widely polymorphic but dimorphic with the exception of block 2. MSP1 alleles have been suggested to be capable of intragenic recombination at the sexual stage, thus generating different genotypes (MSP1 alleles) in the progeny. Although the sites for intragenic recombination between dimorphic MSP1 alleles occur in the N-terminal conserved blocks, recent sequences of laboratory strains have shown recombination in block 4, a variable block. Since recombination in a variable block may increase genetic diversity in MSP1, we have investigated the number and site of recombination in block 4 in natural isolates.

First, we sequenced block 4 of 23 PCR-amplified DNA clones derived from culture-adapted parasites collected in Thailand. Results showed that, in addition

to allelic dimorphic sequences, some isolates had sequence of recombinants between the alleles. The site for the recombination is the same as that reported previously, being at the boundary of residue 365 and 366 (Miller *et al*, 1993). These suggest that recombination in block 4 is confound to one site.

We next searched for the prevalence of block 4-recombinants in an endemic area with parasite DNA recovered from thin blood films on glass slides. PCR amplification with allele-specific primers revealed that block 4-recombinants are not rare in Solomon Islands, indicating that recombination in block 4 indeed increases genetic diversity in MSP1 in the natural population of *P. falciparum*.

A—6

CLONING OF CYTOCHROME OXIDASE III GENE (COIII) FROM *PLASMODIUM VIVAX*

YOH-ICHI WATANABE[1, 2] KIYOSHI KITA[1], SOMEI KOJIMA[1], TAKASHI AOKI[3]
PATRICIA LIM KIM CHOOI[2], TAN SIEW KIAN[2] AND MAK JOON WAH[2]
Department of Parasitology, The Institute of Medical Scdence, The University of Tokyo[1]
Biotechnology Centre, Institute for Medical Research, Malaysia[2]
Department of Parasitology, Juntendo University, School of Medicine[3]

Mitochondrial DNA of *Plasmodium* is a circularly permuted tandem repeat of 6 kb that encodes fragmented both large and small subunit ribosomal RNAs. Mitochondrial genomes of *Plasmodium* have received much interest as they encode some components of the respiratory chain, including cytochrome *c* oxidase subunit I and III (COI and III) anc cytochrome *b* (CYb) as well as rRNAs, and the respiratory chain has been suggested to be a target for antimalarial compounds. However, sequence data for the 6 kb element of human malaria is available only from *Plasmodium falciparum*, although those from murine and avian parasites have been reported. Due to the limited amount of starting material, isolation of pure mitchondrial DNA and its sequence analysis are difficult in the case of other human malaria, such as *P. vivax, P. ovale* and *P. malariae*. We have determined the partial nucleotide sequence of COIII gene encoded on the mitochondrial DNA of *P. vivax* after the successful amplification of DNA using primers designed from the sequence of *P. falciparum*.[1)] In this report, full length sequence of *P. vivax* COIII gene and comparison of the sequence with those of other species are presented.

Alignment of the nucleotides of *P. vivax* COIII with published sequence of *P. falciparum* COIII showed nucleotide homology of 71%, while amino acid sequence comparison gave a homology of 80%. In contrast to the great similarity between two *Plasmodium* species, similarity between *P. vivax* COIII and human COIII (32%) was much lower than that between yeast COIII and human COIII (46%). The invariant glutamate residue that reacts with dicyclohexyl carbodiimide which inhibits proton translocation in coupling site III was found in transmembrne-hilix III. Several deletions and insertions specific for *Plasmodium* enzymes were found, suggesting that tertiary structure of COIII and interaction between the subunits in *Plasmodium* cytochrome *c* oxidase may be different from those of other organisms. The unique feature of the respiratory component found in this study may make this enzyme as a target for specific diagnosis and antimalarial compounds. This study was performed as part of the Institute for Medical Research and Japan International Cooperation Agency Research Project on Tropical Diseases.

1) Patricia Lim K.C. *et al*. Jpn. J. Parasitol. (1995) 44, 40-43

A—7

IL-8 PRODUCTION AND INHIBITORY EFFECT OF THE PERIPHERAL BLOOD MONONUCLEAR AND POLYMORPHONUCLEAR CELLS ON THE *PLASMODIUM FALCIPARUM* IN VITRO PROLIFERATION

JULIETA YURI UCHIDA[1], TADASHI KASAHARA[1,2], AKIRA ISHII[1]
Department of Medical Zoology, Jichi Medical School[1], Division of Biochemistry, Kyoritsu College of Pharmacy[2]

We observed that peripheral blood mononuclear and polymorphonuclear cells are potent inhibitors of *Plasmodium falciparum* (P.f.) proliferation in vitro. We aimed to study the mechanism of how these cells inhibit the P.f. growth and its relationship with the IL-8. P.f. (FCR-3 strain) in continuous culture was used for growth inhibition and IL-8 stimulation experiments. Peripheral blood mononuclear cells (MNC) were isolated by Ficoll/urografin gradient and polymorphonuclear (PMN, 95% neutrophils) by 2.5% gelatine gradient. Growth inhibition tests were performed in 96 wells microplate, where P.f. were added to the MNC or PMN cells, and proliferation was measured 3 days after by incorporation of ^{3}H-hypoxanthine. The inhibitory effect of these cells was proportional to the number of cells added to the culture, and the IC_{50} (inhibitory concentration) for 10^5 infected erythrocytes, was E/T (effector/target) = 0.2 for the MNC and 0.75 for the PMN cells. This means, to obtain 50% inhibition on proliferation of 10^5 parasites, we needed 0.2×10^5 MNC and 0.75×10^5 PMN cells. Moreover, P.f. infected erythrocytes and supernatant of the P.f. culture were potent inducer of IL-8 production not only on MNC but also on PMN. However, blocking of IL-8 with monoclonal antibody did not augment the P.f. proliferation, suggesting that IL-8 is not directly associated with inhibition of P.f. proliferation by these cells. We also investigated the involvement of the Nitric Oxide on the P.f. inhibition, by blocking the Nitric Oxide synthase with N-Monomethyl L-Arginine, but no differences were observed as compared with the control culture.

A—8

GROWTH INHIBITORY EFFECT OF NOVEL PYRROLO [2, 3-d] PYRIMIDINE DERIVATIVES SCREENED BY USING RECOMBINANT *P.f.* DHFR.

TOSHIHIRO HORII[1], REYNOLDS K.B. BROBEY[1], GEN-ICHIRO SANO[1]
FUMIO ITOH[2] AND KAZUYOSHI ASO[2]
Department of Molecular Protozoology, BIKEN, Osaka University[1], Takeda Chemical Industries, Ltd., Osaka[2]

To obtain novel antimalarials, we have screened novel antifolates, pyrrolo [2, 3-d] pyrimidine derivatives by the *in vitro* enzyme assay. We examined 120 of the chemical compounds for their inhibitory effect on the recombinant dihydrofolate reductase (DHFR) of *P. falciparum* produced in *E. coli*. The molar concentrations of the inhibitors that gave 50% inhibition (IC50) of the plasmodial enzyme were estimated alongside with that from the mammalian system when bovine DHFR was used. This enabled the selective inhibition by the inhibitors to be evaluated. We also purified three different *P. falciparum* DHFR with pyrimethamine or cycloguanil resistance for evaluating the cross reactivity of the compounds. Among the tested compounds, one gave 70 fold lower IC50 for *P. falciparum* DHFR than bovine DHFR and inhibited cycloguanil resistant *P. falciparum* DHFR at similar concentration for drug sensitive DHFR. In addition this compound showed appreciable inhibition of the pyrimethamine resistant *P. falciparum* DHFR. These observations were confirmed by the growth inhibition assay of cultural parasites with the drug resistance. The animal trial of this compound by using mouse-*P. berghei* infection system showed the similar efficacy to reduce parasitemia to cycloguanil.

A—9

GENETIC POLYMORPHISM OF THE TNF α PROMOTER REGION IN INDIVIDUALS OF MALARIA-ENDEMIC AND-NONENDEMIC AREA.

AYAKO YOSHIDA[1], MAKOTO ITOH[1], SAUL SAEFAFIA[2] ALBINO BOBOGRI[2]
NATHAN KERE[2], AKIRA ISHII[3], MASATO KAWABATA[4] AND NOBUO OHTA[1]
Department of Medical Zoology, Nagoya City University Medical School[1]
Solomon Institute of Medical Training and Research[2]
Depertment of Medical Biology and Parasitology, Jichi Medical School[3]
International Center for Medical Reseach, School of Medicine, Kobe University[4]

Although detailed mechanisms of the onset of cerebral malaria have not been fully understood, the elevated level of tumor necrosis facter (TNF) α seems to be an important factor determing the susceptibility to cerebral malaria. Genetic polymorphism of the TNF α promotor region has a role in regulation of the cytokine level, resulting significant association between a particular allele (TNF2) and susceptibility to cerebral mararia in Gambia. Guadalcanal Island in Solomon Islands is holo-or hyper-endemic area for malaria, however, the incidence of cerebral malaria in the Island is though to be not so high compared with the case in Africa. Considering the situation, we typed alleles of the TNF α promotor region in individuals of Guadalcanal Island in the hope if we could detect some genetic factors related to susceptibility of cerebral malaria in this Island. We extracted DNA from blood samples of 248 solomonian and 28 Japanese donors. From isolated genomic DNA, 107bp fragment was amplified using primers which was designed to incorporate the polymorphic site into a *Nco I* restriction site. PCR product was digested with *Nco I* and analysed in 2% LMP agarose gel. There is a dimorphism in the TNF α promotor region (TNF 1 and TNF 2). All subjects tested here were typed for TNF 1/TNF 1 homozygote, and the gene frequency was determined as follows; TNF 1=1.00 and TNF 2=0. Difference in frequency of TNF 2 was statistically significant between the Solomonians and Gambian subjects. TNF 2 allele has been suggested to be involved in susceptibility to cerebral malaria, therefore, the possible low incidence of the cerebral malaria in Guadalcanal Island might be related to the absence of the susceptible TNF 2 allele in this Island.

A—10

DIFFERENTIATION OF *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR* DNA FROM CYSTS PRESENT IN STOOL SPECIMENS BY POLYMERASE CHAIN REACTION: ITS FIELD APPLICATION IN THE PHILIPPINES

WINDELL L. RIVERA[1], HIROSHI TACHIBANA[2], MARY ROSE AGNES SILVA-TAHAT[1],
HARUKI UEMURA[1] AND HIROJI KANBARA[1]
Department of Protozoology, Institute of Tropical Medicine, Nagasaki University[1]
Department of Infectious Diseases, Tokai University School of Medicine[2]

It has been established that two distinct species exist within what was originally known as *Entamoeba histolytica*. These are *E. dispar* and *E. histolytica*, for the nonpathogenic and pathogenic forms, respectively. Differentiation of these two organisms is of great clinical importance since they are morphologically indistinguishable and both forms can infect the human intestinal cavity in different degrees. A simple and rapid DNA extraction method which can be used directly on formalin-fixed stool specimens has been developed. The extracted DNA was used for the identification of the existing species in the stools by employing polymerase chain reaction (PCR). Seventy-two (72) randomly-collected stool samples from Brgy. San Antonio, Sasmoan, Pampanga, Philippines were analyzed. Nineteen samples reacted with *E. dispar* primers resulting in the

expected 101-bp PCR products, however, none reacted with *E. histolytica* primers. Furthermore, sensitivity assay suggests that genomic DNA from as low as five cysts can be used as template for PCR. These observations imply that the use of genomic DNA directly extracted from formalin-fixed stool specimens for PCR amplification is a useful tool for obtaining a sensitive and accurate diagnosis that can be applied even in epidemiologic studies.

A—11

DNA DIAGNOSIS OF MALARIA IN VIET-NAM: DETECTION OF OVALE MALARIA AND MALARIAE MALARIA PARASITES

HIROFUMI MIYAKE[1], FUMIHIKO KAWAMOTO[2], OSAMU KANEKO[3]
MASATSUGU KIMURA[4], AND YUSUKE WATAYA[1]
Faculty of Pharmaceutical Sciences, Okayama University[1]
Department of Medical Zollogy, Nagoya University School of Medicine[2]
Department of Medical Zollogy, Osaka City University School of Medicine[3]
and Department of Biophysics, Osaka City University School of Medicine[4]

The cases of ovale malaria or malariae malaria are only sporadically reported in South East Asia. Especially, in the Socialist Republic of Viet-Nam, none of cases of them have been reported officially. We investigated the minor spesies of malaria parasite in Viet-Nam using our DNA diagnosis of malaria, because it is difficult to identify the parasite species morphologically.

We have developed a colorimetric assay "microtiter plate-hybridization" (MPH) to detect amplified DNA of malaria parasites on a microtiter well. This method has high sensitivity and specificity more than microscopic examination. This assay system allows us to detect and identify the four species of human malaria parasites in one assay. Our method utilizes polymerase chain reaction (PCR) and DNA hybridization. A pair of oligonucleotide primers was designed for the amplification of the conserved region of the gene coding for the 18S small subunit of ribosomal RNA. Species-specific oligonucleotide probes were also designed and immobilized on microtiter wells. The target sequence of malaria parasites in human bllod was amplified by PCR and then the PCR-amplified product was captured by the species-specific probe on the microplate well. The biotin-streptavidin system was used for the detection of the PCR products on the wells. Positive samples gave yellow color by the chromogesic reaction.

The epidemiological trials of the DNA diagnosis have been carried out in Viet-Nam from July to August in 1994 and August in 1995. The 209 blood samples (10 μl each) were obtained from donors by finger puncture. The blood samples were diagnosed by microscopic examination in malaria endemic field. They were performed microtiter plate-hybridization in the hospital at Ho Chi Minh City.

As a result, two types of Plasmodium ovale were found; one case has the same 18S ribosomal RNA gene sequence as ovale malaria parasite in West Africa area and the other was new variant of P.o. whose 18S ribosomal RNA gene is different from that of P.o. of West Africa type. Additionally, 5 cases of P. malariae single infection and 2 cases of triple mix infection (P. falciparum, P. vivax, and P. malariae) were also found. We show here the usefulness of this system, more over report the discovery of existence of P. malariae and two types of P. ovale whose 18S ribosomal RNA gene is different in Viet-Nam.

A—12

DISTRIBUTION OF PLASMODIUM MALARIAE IN SICHUAN PROVINCE, CHINA

QING LIU, MIAN ZHOU, MASATSUGU KIMURA*, FUMIHIKO KAWAMOTO AND SHIN ISOMURA
Department of Medical Zoology, Nagoya University School of Medicine, Laboratory of Biophysics, Osaka City University School of Medicine*

Although *Plasmodium malariae* was widely distributed in the middle and south portion of China before 1970's, this species was rarely reported from these regions during the past two decades with only one officially reported case in 1992. Sichuan province, with endemic foci of *P. malariae* in the past, is now considered as a region only has *P. vivax* endemic areas. Dr. Kawamoto, in his visit to introduce the AO stain method in the West China University of Medical Sciences in Sichuan, noticed microscopically the presence of gametocytes of *P. malariae* mixed with *P. vivax* by AO staining of the 2 thin smears from local malaria patients and preserved in the Department of Parasitology, WCUMS. Due to the difficulties of finding the typical band form of *P. malariae*, there were many cases with the species distingiushing problem, said by Dr. XZ, Wang and Dr. SH. Zhou from that department. To confirm the presence of *P. malariae* and its distribution in Sichuan province, an epidemiological survey, as a coorperative work with WCUMS, was conducted in the endemic areas of Junlian County bordering on Yunnan Province and Mingshan County locating at the middle portion of Sichuan.

RESULTS Giemsa stain was used for the diagnosis of malaria in the field by the parasitologists from WCUMS and 66 of 167 local people were positive in malaria. The thin smears of these samples were re-diagnosed and distinguished by AO stain. 26 of the 66 malaria positive cases were diagnosed as mixed infection of *P. vivax* and *P. malariae* with morphologically similarity in band form, schizont and gametocytes to the typical species. Also genomic DNA of malarial parasite from the thick smear of these samples was extracted by following Kimura's method (1995). PCR of 18S rRNA gene was performed with primers in the conserve sequence of *Plasmodium spp.* and nested PCRs with species-specific primers were conducted for the distinguishment of the four human malaria. In 8 cases tested by nested PCR, 2 of them were clearly observed with the amplified DNA band of *P. malariae*, and the other negative cases were highly suspected to be the variants of *P. malariae* or *P. vivax* due to their morphological characteristics resembling *P. malariae*. The sequecing analysis of the target block is performed now. Our results suggested that the *P. malariae* in the endemic areas in China maybe misdiagnosed as *P. vivax*, otherwise, maybe there are some variants of *P. vivax* or *P. malariae* in these areas. This data should be an important reference for the diagnosis and epidemiology of malaria in China.

A—13

DATABASE FOR TRAVELER'S CLINIC

EIICHI OKUZAWA[1], YASUO IKEDA[2] AND TSUTOMU TAKEUCHI[1]
Department of Tropical Medicine and Parasitology, School of Medicine, Keio University[1]
Department of Internal Medicine, School of Medicine, Keio University[2]

We constructed a database of traveler's medicine on a UNIX system (4.2 BSD) to assist consultation at Keio University Hospital. We have been using this system at the traveler's clinic of our hospital. This database is oriented for physicians, and this service is going to be spread to other clinical sections. This system is working all day and accessible from serial line (2400bps, 2 channels) or ethernet (TELNET).

This database includes several sections: geographic areas, microorganisms, symptoms, laboratory tests, drugs and toxins, *etc*.. In the utility of this system, two kinds of usage are basic: The first one is for prevention

of tropical diseases. When we put the name of the area to visit, status about endemic diseases comes out, and ways of prophylaxis are recommended. The other one is for diagnosis and treatment of tropical diseases. When we put the symptom of patients, a list of differential diagnosis appears. Addresses of laboratory tests for definitive diagnosis and drugs for chemotherapy are also available. (Access to the address section is denied except for some users.)

There is a need of constant renovation of the information to follow up practical affairs. We gather all files that carry information of each section in a directory, but we can edit them separately to divide labors. And the index is built automatically after update process. Thus we have reduced maintenance duty.

However more problems are to be resolved. Rules and manners among users are important, because the system provides ways of ordering tests and drugs. But they are not documented yet. Moreover, this system is maintained personally, although it requires a system operator to keep security, a secretary to register users, physicians and researchers to seek information and typists to input it. We have to establish rules for system management and human network of specialists before the number of system users increases.

A—14

POSTGRADUATE EDUCATIONAL AND TRAINING SYSTEM OF TROPICAL MEDICINE IN UK, ESPECIALLY FOR MEDICAL DOCTORS WHO VISIT TROPICAL AREA FOR MEDICAL COOPERATION

KAZUO SUGANE[1] AND GEOFFREY TARGETT[2]
Department of Parasitology, Shinshu University School of Medicine[1] and
Department of Medical Parasitology, London School of Hygiene and Tropical Medicine[2]

Although a lot of young medical doctors in Japan would like to go to the developing countries for the international medical cooperation, there is no good school and hospital in Japan for clinical training of tropical medicine before going to the developing countries. British are much more concerned about tropical diseases compared to Japanese because of large numbers of imported patients with tropical disease and colonial history. U.K. has several good schools and attached hospitals such as London School of Hygiene and Tropical Medicine and Liverpool School of Tropical Medicine. This time educational and clinical training system of tropical medicine for young medical doctors in London School of Hygiene and Tropical Medicine was studied by the cooperation of the school's staff. The London School's system is instructive for considering our own educational and clinical training system of tropical medicine. The school is located at the central part of London and nearly sixty percent are overseas students. It is composed of 4 departments, Dept of Clinical Sciences, Dept of Medical Parasitol., Dept of Epidemiology & Population Sciences and Dept of Public health & Policy. This is the school for postgraduate students and has one year-master's degree course and three year-Ph D course. In addition to these courses the school has special 3 month (Diploma in Tropical Medicine & Hygiene) and 1 year-master's course (MSc Infection & Health in Tropics) for medical doctors who are interested in tropical medicine. These training course are substantial. Attached hospital (Hospital for Tropical Diseases) was built in 1951 and new hospital will be built in the near future at the different place. These are in-patient facilities for 30 patients, and approximately 1,200 patients are treated each year. Over 300 of these patients suffer from malaria, while other important diseases include diarrhoea, filariasis, schistosomiasis, leprosy, viral hepatitis, typhoid fever, amoebic liver abscess, leishmaniasis and hydatid disease. There are also approximately 900 out-patient sessions per year. The hospital is used for clinical training of young medical doctors in MSc and Diploma course.

A—15

A HISTORICAL REVIEW OF EUROPEAN TRADITIONAL DOCTORS AS A BACKGROUND OF SO-CALLED WITCH DOCTORS IN KENYA (NOTE)

JUN MAKI[1], YOICHI ITO[1], YOSHIKI AOKI[2], YASUNORI FUJIMAKI[2] AND YOSHINORI YASUDA[3]

Dept. Parasitology, Kitasato University School of Medicine, Sagamihara, 228 Japan[1]
Institute for Tropical Medicine, Nagasaki University, Sakamoto-cho, 852 Japan[2]
International Research Center for Japanese Studies, Kyoto, 610-11 Japan[3]

When Maki *et al.* (1995, Jpn. Trop. Med. Hyg. *23*, 78) surveyed traditional medicine in Kenya in the hope of finding useful herb medicine for the treatment of human filariases, they noticed that the specialists who are engaged in traditional medicine in the country were often called "witch doctors" especially by people from developed countries including Western researchers. Some scientists in Kenya were against the word "witch doctors", saying that the expression originated in a kind of bias. They asserted that there are no witch doctors in Kenya as European people imagine. There used to be witch doctors in Europe. It is said that their various kinds of practices date as far back as medieval or ancient times. What were European witch doctors like? We studied it bibliographically in order to conclude whether the usage of witch doctors is appropriate for traditional doctors in Kenya or not.

According to Ueyama (1993, Witches and Christianity, Jinbun-shoin) and Schmölzer (1994, Phänomen Hexe-Wahn und Wirklichkeit im Lauf der Jahrhunderte, translation published by Hakusui-shya), the traditional witch doctors in Europe had worked for the maintenance of the health of people living in villages, and the treatment of their illness. They had a good mastery of various kinds of crude drugs for these purposes. The drugs used in those days are classified into three categories, those of animal and plant origin, and minerals. Before the Christianity was brought into the region of pagan societies, the witch doctors had been respected by their village people. However, the dignity of the traditional doctors were humiliated by the invading powers with the help of newly started universities. Although no information has been available as to the possible criticism against the custom of using animal-origin drugs like feces (Maki *et al.*, Jpn J. Parasit., in press), folk medicines like medicinal plants used by the traditional doctors were looked down upon (Ueyama, ibid; Schmölzer, ibid.). The criticism was so severe that they were considered to be a witch. The custom of the use of spring water (mineral medicine) which had been worshiped in the pagan villages in Europe was also subjected to the severe criticism by the new invading powers above mentioned.

A large number of them were sent to witch trials to be persecuted. It is probable that their resistence lasted for centuries. The custom of using feces as medicine possibly played a role in the resolution of life cycle of parasites in 19th century. It is a matter to be studied further (Maki *et al.*, ibid.). The traditional use of herb medicine led to the formation of a basis for leading substances of modern medicine. The traditional worship for spring water seems to have survived the criticism (Maki *et al.*, ibid.).

It seems that the idea of witch doctors persecuted resides in the popular mind in the European subconsciously. In conclusion the expression "witch doctor" is not appropriate in Kenya.

A—16

CURRENT SITUATION OF MEDICAL CARE OFFERED TO JAPANESE STAYING OVERSEAS

ATSUO HAMADA, MASAMI MIZUE, MASANOBU KAIDO, YUKIO FUJI, KANAMETSUKUI
SHIGEYUKI NISHIMURA AND TETSUO NISHIKAWA
Japan Overseas Health Administration Center

To know the current situation of medical care offered to Japanese staying abroad, we administered questionnaires to Japanese staying abroad who had medical care in the area.

METHOD We send questionnaires to Japanese Societies in 14 cities of different countries and asked members whose family had medical care there to answer them. In total 514 questionnaires were collected to be analyzed: 318 of them were from eight developed countries and 196 of them were from six developing countries.

RESULT Many visits to overseas medical facilities by Japanese were because of acute diseases with clear symptoms such as respiratory diseases and gastrointesitnal diseases. Fewer visits were because of chronic diseases such as circulatory, metabolic, and endocrine diseases with less clear symptoms. Many answered they chose their medical facility because there was no language barrier, because their friend recommended it, or because it was near their home or working place. The result of tratment was relatively good both in developed and developing countries. Informed consent was sought less often in developing countries than in developed countries. In developed countries, respondents said medical care was expensive. In developing countries, respondents said it was reasonable.

CONCLUSION It appears that problems were not so big when Japanese clients visited overseas hospitals in the case of acute diseases. We now need to find out why they don't see a doctor so often in the case of chronic diseases and try to urge them to go see a doctor even in the case of chronic diseases.

A—17

PROBLEMS OF A FIELDWORK IN NEPAL

EIKO KANEDA
Department of Liberal Arts, Nagasaki University

Recently scientists and field workers have directed more attention on Medical Anthropology. In this field, it is necessary that examiners visit the target places by themselves and/or live among the natives to study their society. There are many factors to be taken into consideration in fieldwork. Particularly in the developing countries, it is hard for examiners to predict what is going to happen when they enter the society. The purpose of this paper is to examine this kind of problem based on an experience in Nepal during a physique and living-time study of Nepalese women.

The target places in Nepal were Tirku village (2,570m above sea level), Thuribidi village (1,025m), and Johnapur village (170m). The subjects were women from the ages of 20 to 40 years old. The items of the measurement were their height, weight, chest, sitting height, blood pressure and grasping power. In addition to this, a living-time study was conducted. The following are some examples of the problems that occurred:

1) The difference between examining and medical treatment was not clear among the informants. Sometimes the examiner was regarded as a medical doctor, and medicine was requested by the informants.
2) Some people could not stand on the weight scale with their feet together.
3) Because of the Hindu concept of uncleanliness, some informants rejecoed the measurement of oral temperature.
4) Sometimes the examiner was not able to eat any of the food due to the difference of hygienic senses from those of the natives.

Considering many other incidents along these lines that happened during the fieldworking, we can classify

the problems as follows:

1) maladjustment to the measurement itself; over self-defence, over expectation.
2) maladjustment to the apparatus and the measuring method.
3) physical maladjustment.
4) social and cultural maladjustment.
5) problems based upon the readiness of the examiner.

Some suggestions for dealing with these problems are to be discussed during the session.

A—18

ASSISTANCE FOR RECONSTRUCTION OF POST-CONFLICT REGIONS: LOCAL NGOs ROLES ON HEALTH IN THE WEST BANK AND THE GAZA STRIP

AYAKO SHIDA[1], TAKEFUMI KONDO[2], TSUTOMU TAKEUCHI[2]
Department of Tropical Medicine and Parasitology, School of Medicine, Keio University[1]
Department of Public Health, School of Medicine, Keio University[2]

This presentation is to discuss roles of local NGOs (Non-Governmental Organization) on health in the West Bank and the Gaza Strip as a case study for reconstruction assistance for health services in post-conflict regions, based on interviews with Palestinian Authority, UN agencies, international and local NGOs' staffs in the regions from March to April 1995.

The West Bank and the Gaza Strip had been military-occupied by Israel since 1967 up to 1994 when they partially started self-autonomy. The health conditions have been situated on lower middle income countries, and the health institutions have been fragmentedly administrated by 4 sectors; governmental, a UN agency for Palestinian refugees called UNRWA, NGOs and private.

It is difficult to generalize characters and roles of local NGOs, different from size or quality of services, however, in average, there were more than 40 patients per doctor at an NGO clinic per day in the Gaza Strip while 10 to 20 patients in the West Bank. Nevertheless, it is not rare that a local NGOs clinic is the only health service provider in a rural village scattering in the West Bank. In addition, NGO sector provides services with reasonable charges (average US$ 1.5 per consultation) while governmental ones work within a health insurance system which more than 70% of total population cannot take advantage.

Since 1993, due to decline of socio-economic circumstances affected by the restriction against transportation of Palestinian people, not a few of NGOs were obliged to close their clinics, which governmental or UNRWA sector has difficulties to take over with lim ited financial source. It means that Palestinian people's accessibility to health institutions was declined after the self-autonomy in the West Bank and the Gaza Strip.

The presentation emphasizes an importance of local NGOs' roles in post-conflict regions with their long year experience and human relationship in local communities although their political characters and/or lack of transparency for fiancee would become obstacle against practice of international cooperation activities. However, it is impossible to disregard their roles, for the main objective of reconstruction assistance for post-conflict regions is to utilize local resources and strengthen local capacity.

Government of Japan is to introduce a new item called "reconstruction and development assitance" for post-conflict regions from the fiscal year of 1996. It is hereafter necessary to discuss the ways of management to cooperate with local NGOs in rehabilitation and reconstruction assistance for post-conflict regions.

A—19

COMPARATIVE LINKAGE MAPS FOR THE MOSQUITOES, *AEDES ALBOPICTUS* AND *AEDES AEGYPTI*, USING RESTRICTION FRAGMENT LENGTH POLYMORPHISMS, RFLP

AKIO MORI , DAVID W. SEVERSON, V.A. KASSNER AND BRUCE M. CHRISTENSEN
Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison

Aedes albopictus and *Aedes aegypti* are members of the mosquito subgenus Stegomyia. Both species are known as the vector for the transmission of dengue fever virus in areas of the tropics. *Ae. aegypti* is capable of transmitting filarial parasites, *Brugia malayi, B. pahangi* and *Dirofilaria immitis*. *Ae. albopictus* also plays the vector of *D. immitis*. Localization of genes susceptible to these pathogens can be accomplished through linkage analysis with previously mapped genetic markers in *Ae. aegypti*. On the other hand, only few genetic markers available to this study are reported in *Ae. albopictus*. Restriction fragment length polymorphisms (RFLPs) represent a class of genetic markers that permit the development of detailed genetic maps from a limited number of crosses. The number of RELP is virtually unlimited, and they have no effect on phenotype, are condominant, and exhibit Mendelian inheritance. We reported construction of a saturated RFLP linkage map for the *Ae. aegypti* already using *Ae. aegypti* cDNA or genomic DNA clones. Then construction of comparative linkage map for *Ae. albopictus* was carried out based on these cDNA clones from *Ae. aegypti*. Nearly all *Ae. aegypti* probes hybridized to *Ae. albopictus* genomic DNA at high stringency. For 18 RFLP markers tested, the linkage group and linear order appears to be identical for the two species. Seventy eight percent of the loci tested exhibited significant deviations from the expected segregation ratio in at least one of the test crosses. An excess of heterozygote genotypes was recovered with most loci. This probably reflects the effects of lethal loci on survival of F_2 progeny homozygous for the parental genotypes. These results demonstrate that comparative linkage maps based on common DNA markers provide a basis for rapidly developing linkage maps for various mosquito species, and the opportunity to examine the significance and function of orthologous quantitative trait loci associated with mosquito vector competence for disease transmission.

A—20

EFFECT OF CLEARING COVER OF VEGETATION ON THE BANKS OF A SLOW RUNNING STREAM ON LARVAL DENSITY OF *ANOPHELES MINIMUS* THEOBALD (DIPTERA: CULICIDAE)

MASAHIRO TAKAGI[1], YOSHIO TSUDA[1], WANNAPA SUWONKERD[2],
AKIRA SUGIYAMA[3] AND YOSHITO WADA[1]
Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University[1],
Malaria Center 2, Division of Communicable Diseases Control, Ministry of Public Health, Thai Government[2],
Department of Public Health, Faculty of Domestic Science, Nagoya Wowen's University[3]

The effect of clearing cover of vegetation over the surface of water on the larval density of *Anopheles minimus* was investigeted in a slow running stream in a shallow valley of north Thailand. The stream was divided into 2 blocks each measuring 300 m long, and at one of the 2 blocks all vegetation covering the water surface was cleared (experimental block), while the other with sparsely covered by vegetation was left without any treatment (control block). After the clearing, the average larval density in the 2 blocks was monitored for 1 yr together with several environmental parameters. Although the larval density little changed in the control block, it increased within 0.5 month after the clearing in the experimental block. By 6 mo after the clearing, the vegetation recovered, and became to cover the water surface again. The risk to facilitate the

infestation of *A. minimus* by a careless deforestation was emphasized.

A—21

FEASIBILITY OF USING OUTDOOR LIGHT-TRAPPING IN MALARIA VECTOR SURVEILLANCE

MOTOYOSHI MOGI[1], ICHIRO MIYAGI[2], TAKAKO TOMA[2]
Department of Microbiology, Saga Medical School[1]
School of Health Sciences, Faculty of Medicine, University of the Ryukyus[2]

Human-bait collection, which has been established as a standard method for malaria vector surveillance, has a disadvantage in that collectors are exposed to the risk of malaria infection. Therefore, feasibility of using light traps in malaria vector surveillance has been examined in Africa and Asia; the results indicate that indoor light trap collection can be used as a substitute for human-bait collection, because vector species composition and age structure were similar between the two methods. However, indoor operation of light traps may be uncomfortable for people sleeping there. We examined the age structure of *Anopheles subpictus* (a suspected vector of malaria in villages along the coast of Halmahela, Indonesia) collected by a light trap (6W BL lamp) operated outdoors. The females attracted to cattle included individuals with small-sized ovaries and those with large-sized ovaries. The size of small ovaries corresponded to that of unfed females which emerged from larvae collected in the field and were reared in the laboratory with sugar solution. In contrast, the females collected by outdoor light traps exclusively consisted of individuals with large-sized ovaries. Questions arising from these facts are (1) that *Anopheles subpictus* females in this area may require at least two blood meals for egg production, and (2) that outdoor light traps may collect aged females more efficiently than host-baited collection. The latter point needs to be confirmed by future studies, because collections biased to aged females could be an advantage if the purpose of malaria vector population surveillance is limited to know the intensity of relative risks of malaria infection.

A—22

USAGE OF PERMETHRIN-IMPREGNATED BED NETS (PBN) FOR THE CONTROL OF ISLAND MALARIAIN VANUATU

AKIRA KANEKO[1], GEORGE, K. TALEO[2] FUMINORI AOYAMA[1], CHIHIRO SHIRAKAWA[3] AND TAKATOSHI KOBAYAKAWA[1]
Department of International Affairs and Tropical Medicine, Tokyo Women's Medical College[1], Malaria Division, Vanuatu[2], the Graduate University for Advanced Studies[3]

Vanuatu in Melanesia consists of about 80 islands with meso to hyper malaria endemicity. The authors were involved in the WHO Malaria Control Project here from 1987 to 1994. Early diagnosis and treatment of malaria patients at all level of health care delivery to prevent malaria mortality is the principle element of anti-malaria measure. While malaria incidence has remained high, PBN has been used with PHC approach to reduce malaria morbidity. In Vanuatu the first net was treated by permethrin at Epule Village of Efate Island on May 1988. Up to the end of 1992, a cumulative total of 30,942 bed nets were distributed to protect 39,962 population in 9 islands (26.7% of the total population). It is remarkable a ratio people/net is very high in Vanuatu (1 net for 1.29 people), while the average in other countries is 1 net for 2.2 people. Bed nets are impregnated with permethrin once every year in villages. Prior to treatment, all nets are marked the date and place of treatment on with an indelible pen. Dipping and dripping is carried out by village volunteers under the supervision of a district malaria supervisor. Used nets are washed on the day before treatment. The

target dose of permethrin is 500 mg/m². In our observations to get this dosage, 2.0% permethrin solution must be prepared from the original permethrin 50% EC for Manila nets, but 1.43% for Thai nets. For this purpose workers are instructed to use a simple calculation table to get a proper permethrin solution. An amount of permethrin and water needed to treat bed nets is measured in a calibrated plastic cylinder and poured into a large plastic bowl. People dip and drip their own nets and make the nets dmied under shadow on top of a bare mattress. After treatment, villagers wath their hands with soap and water provided by the district malaria supervisor, who makes record of number of bed nets, actual dosage of permethrin, etc. A operational manual of permethrin impregnation of mosquito nets in villages was produced. We give nets to mothers and small children without any charge, but charge adults a price for a net and students a half price for that. Changes of parasitological parameters were monitored in Emae from 1983 to 1990, where intervention by impregnated mosquito nets was carried out in 1988. Those in Nguna Island without any intervention were also monitored as control. Both islands showed outbreak of malaria in 1985 and natural subsidence in 1988. Although the next outbreak occurred in 1990 in Nguna, malaria situation was continuously suppressed in Emae. In Aneytyum, the southernmost inhabited island, mass drug administration and PBN almost eliminated malaria parasites in 1991 and has continuously maintained the situation. Major operational problems are difficulty of transportation, management of impregnation in communities, actual usage of bed nets by villagers after distribution etc.

A—23

MALARIA VECTOR CONTROL STRATEGY IN THAILAND FROM SUCCESSFUL CONTROL MEASURES

TOZO KANDA et al.
Japan Association for Tropical Medicine, Tokyo, Japan

Two highly endemic communities with multi-drug resistant falciparum malaria were selected for entomological control measure. The populations of those communities were 202 and 206 against other surrounding ones respectively 805 in Village 6 and 853 in Vill. 8, Patavee, Makham, Chanthaburi Province. Other control measures were similarly performed to both, intervention and control since 1991 to date. The malaria cases were extremely yearly decreased attaining less than yearly 5 case during past 5 years without death and severe one. The control measures applied were Kanda's sound trapping at breeding site up to the vectors disappear, application of IGR (5 ppm of pyriproxyfen) for larvae and pupae in their breeding streams or 3 monthly intervals or releasing of predator (guppy fish). Other control measures were similarly performed as other control communities such as residual spray, impregnated bed-net method adopting etofenprox instead of DDT in biting site yearly twice.

The vectors, *Anopheles dirus, An. maculatus, An. minimus* reduced in cumulative number traped 5 days from 135 to 6 with 5 trapping in 22 weeks follow-up, while other species remained unaffected 762/792. 32 traps were used for this control measure. Falciparum malaria patients were reduced from 45 to 1 (with 2 of vivax malaria) in the integrated measure and from 127 to 39 in the DDT resudual spray method. Releasing of predator and applicatdon of pyriproxyfen (IGR) were performed in running streams at relative low endemic area, Tab-Kwang District in Saraburi Province. Malaria case was disappeared within the year commenced and is now keeping malaria free.

For monitoring of the vector caching mosquitoes by using Kanda's sound trap system at breeding site and double net human bait using dry ice at the entrance of malaria case residing house were very much effective to know vector prevalence and interruption of the infection. Civilengineering of land for improvement of breeding sites were also jointly adopted to these control measures according to request. Along to the border the control measures in Chanthaburi Province multi-drug resistant falciparum malaria is now extremely decreasing and the transmission has been interrupted with exception of imported case.

A—24

INVESTIGATION ON THE INFECTION OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* SPOROZOITES IN *Anopheles sinensis* CAPTURED AT NEW TOKYO INTERNATIONAL AIRPORT

MASATO ITOI[1], KISEKO KAMEI[2], TAKESHI KURIHARA[2]
Nagoya Airport Quarantine Station[1]
Department of Parasitology, Teikyo University School of Medicine[2]

Ninety-one female mosquitoes of *Anopheles sinensis* were captured at New Tokyo International Airport compound in 1994. Adult mosquitoes were collected using sweeping nets and light traps. The larvae also collected using a ladle and pipette.

In addition, cabin of arrival aircrafts from malaria endemic areas, mostly tropical Asian countries, were also inspected. We surveyed 2% of those 9,000 aircrafts, belongs to 51 air companies, with sweeping nets and aspirators, but no anopheline mosquitoes were found during the present survey. Samples identified as *Anopheles sinensis* were stored by drying until examination, and were examined for the existence of CS protein (circumsporozoite protein) in their salivary glands using ELISA devised by Dr. R.A. Wirtz. Laboratory bred *An. stephensi* were used as negative control. Neither *Plasmodium falciparum* nor *P. vivax* CS protein were detected in 91 females of *An. sinensis*.

At New Tokyo International Airport, aircrafts coming from malaria endemic area exceeds 9,000 in a year. In this situation, it might be difficult to say that Japan is free from malaria. During the present investigation, the mosquitoes infected with *Plasmodium* was not detected, but further investigation should be required. We will continue the survey as a part of the malaria surveillance, and also expand the mosquito examination to the other international airports all around Japan in near future.

A—25

PREVALENCE OF MALARIA AND ITS RELATIONSHIP TO ANEMIA, NUTRITION, AND HAPTOGLOBIN POLYMORPHISM IN THE SOLOMON ISLANDS

YUTAKA MIZUSHIMA[1], HIROMI KATO[1], HIROSHI OHMAE[2], TOSHIAKI TANAKA[3]
ALBINO BOBOGARE[4] AND AKIRA ISHII[5]
First Department of Internal Medicine[1], Second Department of Internal Medicine[3]
Toyama Medical and Pharmaceutical University, National Medical Center[2]
Solomon Islands Maralia Research Institute[4] Department of Medical Zoology, Jichi Medical University[5]

Prevalence of malaria and its relationship to anemia, nutrition, and haptoblobin polymorphism was examined on Guadalcanal Island in the Solomon Islands in 1993. A total of 506 residents participated in this study. The slide positive rate for malaria was 54% (275/506) in all ages, with a high of 79% for children aged 4-6 years. *Plasmodium falciparlum* was the most common species (52%), followed by *P. vivax* (29%). Body mass index was lower in Solomon Islanders than for the Japanese population up to 15 years old in both genders. Mean values for serum insulin-like growth factor-1 (IGF-1) were also lower in Solomon Islanders in children under 18 years old. The hemoglobin distribution curves were almost identical in the malaria-positive (P(+)) and -negative (P(−)) groups. The percentage of cases with less than 80mg/dl of blood glucose and those with less than 50mg/ml of IGF-1 were higher in the P(+) group than for the anti-malaria drug-untreated malaria-negativj ((P−)D(−)) group. In cases under 25 years old, 17% (19/11) were ahaptoglobinemic (Hp0), and no association was observed between Hp0 and parasitemia. These results suggest that low blood glucose and low IGF-1 levels may have some relationship with the malaria infection, but the Hp0 may not in the Solomon Islands.

A—26

HEMOGLOBINOPATHY SCREENING AND ITS ANALYSIS AMONG MALARIA ENDEMIC POPULATION IN SOLOMON ISLANDS

MASATO KAWABATA[1], TERUO HARANO[2], KEIKO HARANO[2], AKIRA ISHII[3]
JULIETAYURI UCHIDA[3], NOBUO OHTA[4] AND RYUTARO OHTUKA[5]
ICMR, Kobe University School of Medicine[1], Department of Biochemistry, Kawasaki Medical School[2]
Department of Medical Biology and Parasitology, Jichi Medical School[3]
Department of Medical Zoology, Medical School, Nagoya City University[4]
and Department of Human Ecology, Faculty of Medicine, Tokyo University[5]

To study the distribution of hemoglobinopathy in Southwest Pacific malaria endemic areas, 186 red blood cell samples from Solomon Islands and 212 from Papua New Guinea (PNG) were analysed by isoelectric focusing (IEF) and high permormance liquid chromatography (HPLC). Bood samples examined were collected and washed in Guadalcanal Province, Solomon Islands and Manus Province, PNG, and were shipped to Japan under frozen condition. In solomon Islands where malaria endemicity is holoendemic, 22 cases (11.8%) exhibited tree types (A, B and C) of abnormal Hb. Numbers of abnormal Hb cases in three different type (A, B and C) groups were 4, 16 and 2 respectively. In PNG, however, where malaria infection rate was less than 2%, none of red blood cell samples showed abnormal Hb.

Structural analysis of these Hbs was performed by an amino acid analysis of abnormal peptide and a nucleotide sequencing of the defective globin gene. Type A Hbs had an α chain anomaly and their contents were aproximately 36 to 46% of the total Hb. They had an amino acid substitution of Ala→Asp, identifying to Hb J-Tongariki, and a nucleotide change from GCC to GAC at the 155th position of the α2-gene. Type B Hbs, which had a β chain anomaly and were accompanied by an extra brownish Hb band, had a substitution of Lys→Glu and a mutation of AAA→GAA at β 66, identifying to unstable Hb I-Toulose. Type C Hbs had tree different types of abnormal Hb composed of normal α and β, α-J-Tongariki and β-I-Toulose chain, respectively. Infection with *Plasmodium* based on microscopical and/or PCR examination was found in one case of type A group, eight of type B and none of type C. The possibile relationship between the resistance to malaria infection and the patients with abnormal Hb was discussed.

A—27

MOLECULAR ANALYSIS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS FOUND IN THE SOLOMON ISLANDS

AKIRA HIRONO[1], SHIRO MIWA[1], HISAICHI FUJII[2], ALBINO BOBOGARE[3]
NATHAN KERE[3] AND AKIRA ISHII[4]
Okinaka Memorial Institute for Medical Research[1],
Department of Transfusion Medicine, Tokyo Women's Medical College[2],
Medical Training Research Institute, Solomon Islands[3],
Department of Medical Zoology, Jichi Medical School[4]

One of the complications in the malaria chemotherapy is drug-induced hemolytic anemia in glucose-6-phosphate dehydrogenase (G6PD) deficient subjects particularly by primaquine. It is quite important to screen G6PD deficient subjects and to identify the type of variant enzymes they carry before starting the chemotherapeutic control. To clarify the molecular features of G6PD deficiency in the Solomon Islands, we have performed polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) and sequence analysis of G6PD variants in Guadalcanal Island.

A total of 453 subjects from villages near Honiara on Guadalcanal Island were screened for G6PD deficiency by the formazan ring spot test. Twenty-seven

individuals (21 males and 6 females) were found to be markedly deficient in red cell G6PD activity. Using non-radioisotopic PCR-SSCP analysis, we identified two types of mutants in the 27 G6PD deficient subjects. One mutant bore a single base substitution of C to T at nucleotide 1360 predicting an Arg to Cys substitution at residue 454 that was already reported to cause a class II variant G6PD Maewo (Union). The other mutant carried two missense mutations: an A to G substitution at nucleotide 99 predicting an Ile to Met change at amino acid 33 in addition to the common 1360 C→T mutation. The 99 A→G was unique and caused a new variant G6PD Honiara in combination with the 1360 C→T. The finding of the same mutation in G6PD Honiara as was found in G6PD Maewo strongly suggests that the 99 A→G occurred in an individual with G6PD Maewo.

G6PD Maewo is one of the most frequent G6PD variants, which has been found in Philippines, Thailand, Laos, Vanuatu, the Southern China and the Southern Europe. Because subjects with G6PD Maewo are known to be highly sensitive to primaquine, screening for G6PD deficiency should be indispensable before starting the primaquine therapy in the malaria endemic areas.

A—28

ANTI-*TRYPANOSOMA CRUZI* ACTIVITY OF EXTRACTS FROM NATIVE MEDICINAL PLANTS USED IN GUATEMALA FOR PROTOZOAL INFECTIONS (MINIREVIEW)

ARMANDO CACERES[1], BEATRIZ LOPEZ[1], JUN MAKI[2] AND ISAO TADA[3]
Faculty of Chemical Sciences and Pharmacy, University of San Carlos, Guatemala[1]
Dept. Parasitology, Kitasato University School of Medicine, Sayamihara 228, Japan[2]
Dept. Parasitology, Kyushu University School of Medicine, Fukuoka 812, Japan[3]

Chagas' disease is endemic to South and Central American countries including Guatemala. It is an ailment of public importance in rural areas. Unfortunately, it has been rather an orphan disease in spite of the fact that Guatemalan people have been suffering from this disease. Few actions for its control have been undertaken and no chemotherapy is available in the country so far. Drugs from indigenous plants are expected to play a role in the treatment of various kind of diseases, so there would hopefully be a way to treat Chagas' disease with plant extracts with proven activity and safety (1). Although no medicinal plants have been reported to be useful for the treatment of this disease. This communication describes the preliminary screening to find plants with such activity.

Plants used for the treatment of protozoal disease (amebiasis, leishmaniasis, malaria and vaginitis) were selected based on ethnobotanical and literature information (2, 3). Thirteen plants were collected, shade-dried, extracted with dichloromethane, ethanol and water, concentrated by rotavapor or lyophilization and stored in a vacuum dessicator. In vitro procedures were standardized for epimastigotes (Tulahuen strain) cultured in LIT medium, and trypomastigotes cultivated in L cells for 2-3 months at 37°C and separated in a CM-cellulose column. Protozoa were challenged with 1 mg/ml of extract in microwell plates, counted in an hemocitometer in conventional or inverted microscope, and compared with positive (gentian violet or allopurinol) and negative controls. In vivo procedure consisted of infection of 5 mice, oral administration every other day of 500 mg/ml of the extract, bleeding by day 7, 14 and 21, microscopical evaluation, and comparison with controls. Toxicity was evaluated by *Artemia salina* in microplate and analyzed by a Finney computer program to determine LC50. Oral acute and intraperitoeal subacute toxicities were evaluated by standard methods.

Two plants showed in vitro and in vivo activity (*Neurolaena lobata* and *Solanum americanum*) and three were positive only in vivo (*Acalypha guatemalensis, Croton guatemalensis* and *Tagetes lucida*). Toxicity was demonstrated in one of the active extracts. Cooperation with Japanese researchers for further chemical elucidation of active principles is being encouraged (3).

REFERENCES 1. Maki & Caceres (1993) JICA report pp. 18; 2. Caceres et al. (in preparation); 3. Caceres (1995) Kitasato Medicine, 25 (in press)

The authors wish to thank the technical and financial support of DIGI/IIQB of University of San Carlos (Vivian Matta, Sonia Gonzalez, Nancy Garcia, Aura Padilla and Federico Nave), JICA (Drs. Y. Tabaru and T. Yanagi), Kitasato University School of Medicine

(Prof. Y. Ito), and Farmaya Laboratory.

A—29

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF 13 NATIVE PLANTS USED IN GUATEMALA FOR THE TREATMENT OF PROTOZOAL INFECTIONS (MINIREVIEW)

ARMANDO CACERES[1], SONIA GONZALEZ[1] ISAO TADA[2] AND JUN MAKI[3]
Faculty of Chemical Sciences and Pharmacy, University of San Carlos, Guatemala[1]
Dept. Parasitology, School of Medicine, Kyushu University, Fukuoka 812, Japan[2]
Dept. Parasitology, Kitasato University School of Medicine, Sagamihara 228, Japan[3]

Mesoamerica is the region where the Maya empire flourished for several centuries, and a vast heritage is still present. Diseases due to bacterial, fungal and protozoal infections are common ailments of public importance in the rural areas. Drugs from indigenous plants are expected to play an important role in the treatment of these infections(1). However, the traditional heritage is disappearing with the natural resources due to lack of systematization of this knowledge and severe deforestation in recent years.

Thirteen native plants traditionally used for the treatment of protozoal diseases such as malaria, leishmaniasis, vaginitis and dysentery were selected based on ethnobotanical and literature information(2, 3). They were collected, shade-dried and extracted with dichloromethane, ethanol and water, concentrated by rotavapor or lyophilization and stored in a vacuum dessicator.

Activity against bacteria (*Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus*), yeast (*Candida albicans* and *Cryptococcus neuformans*) and fungi (*Aspergillus flavus* and *Microsporum gypseum*) were screened in vitro by dilution procedures in Muller Hinton or Sabouraud agar plates (10 mg/ml).

Preliminary results indicate that from 13 plants screened, seven were active against bacteria, five against yeasts, and six against *M. gypseum*. The best solvent for the extraction of activity was ethanol. Most interesting plants with activity at <5 mg/ml are *Acalypha guatemalensis, Byrsonima crassifolia, Neurolaena lobata, Tagetes lucida* and *Smilax lundellii*. The presentation 3 showed the microbial spectrum results, the minimal inhibitory concentration of the extracts, and a preliminary screening of the phytochemical composition.

Possibilities for further cooperation in chemical isolation and elucidation of the active principles between researchers from Guatemala and Japan is encouraged(3).

REFERENCES 1. Maki & Caceres (1993) JICA report pp. 18; 2. Caceres et al. (1995) Enfermedades Tropicales en Guatemala (in press); 3. Caceres (1995) Kitasato Medicine, 25 (in press)

The authors wish to thank the financial and technical support by DIGI/IIQB of University of San Carlos (Beatriz Lopez, Aura Padilla, Federico Nave and Fabiola Meza), Iberoamerican Program for Science and Technology for Development (CYTED), Farmaya Laboratory and JICA (and Prof. Yoichi Ito, Kitasato University Dr. Yoichiro Tabaru, supervisor).

A—30

ELECTRON MICROSCOPIC STUDY OF PATIENTS WITH CUTANEOUS LEISHMANIASIS TREATED WITH MEFLOQUINE

MOTOYOSHI MARUNO[1], ATSUSHI HOSOKAWA[1], SHIGEO NONAKA[1], YOSHIHISA HASHIGUCHI[2]
Department of Dermatology, Research Center of Comprehensive Medicine, Faculty of Medicine
University of the Ryukyus[1], Department of Parasitology, Kochi Medical College[2]

Introduction

Sixteen patients with cutaneous leishmaniasis were

treated with the administration of mefloquine in Equador, South America in 1995. As a result, nine of them had been cured within 3 weeks of the administration; six of them within 4-6 weeks; one patient after 8 weeks. No specific adverse reactions had been observed during the treatment. The mechanism how mefloquine affects leishmanial protozoans is unknown. It is speculated, however, that mefloquine may inhibit amastigote-macrophage interactions in leishmaniasis with the same manner as in malaria. In this study, we report the results of electronmicroscopic as well as lightmicros copic observetion of the ulcer specimens from a 41-%jf8-old male patient treated with mefloquine.

MATERIALS AND METHODS A total of four specimens were taken from the margin of the ulcer sized 35×25mm with one specimen at a time, at four different times such as before the treatment, 2 weeks, 4 weeks and 6 weeks after the treatment. Each specimen was cut into two pieces: one for lightmicroscopic and the other for electronmicroscopicexaminations, respectively. The former was stained with two different methods: hematoxylin-eosin staining and immunohistochemical staining with anti-asialo GM1 antibody.

RESULTS The specimen taken before the treatment showed epidermal hyperplasia and mild exocytosis of lymphoid cells, while that taken 4 weeks after the treatment revealed the almost normal epidermis. The former also showed dense infiltration of lymphoid cells and macrophages throughout the dermis together with leishmanial protozoans both inside and outside of the macrophages. In contrast to this, the latter showed localized infiltration of lymphoid cells and giant cells around the vessels with few leishmanial protozoans.

Immunohistochemical study revealed the following: the specimen before the treatment showed the anti-asialo GM1 antibody positive cells throughout the dermis, whereas that taken 4 weeks after the teatment only showed the anti-asialn GM1 antibody negative cells in the dermis.

Electronmicroscopic study revealed the following: in the specimen before the treatment, the macrophages attached with each other, and the organelles within those were well developped. In the specimen 4 weeks after the teatment, however, the intercellular spaces between the macrophages enlarged, and the cytoplasms of those showed vacuolar degeneration. The plasma cells also showed enlargement of the intercellular spaces in the specimen 4 weeks after the treatment. Enlargement of the rough endoplasmic reticulums of the plasma cells were showen in the specimen before the treatment, but there were no equivalent findings to this in the specimen 4 weeks after the treatment.

DISCUSSION We consider that the changes shown in the macrophages and the plasma cells before and after the treatment may be caused as a result of healing of leishmaniasis. Further clinical and experimental investigations are needed.

A—31

CLINICAL COURSES OF IMPORTED MALARIA CASES FOLLOWING TREATMENT WITH HALOFANTRINE

SHIGEYUKI KANO[1], GOHTA MASUDA[2] AND MAMORU SUZUKI[1]
Department of Parasitology, Gunma University School of Medicine[1] and Department of Infectious Diseases, Tokyo Metropolitan Komagome Hospital[2]

Now, the reported annual number of imported malaria cases has increased to not less than 100 in Japan. Thus, the importance of instituting prompt diagnosis and proper treatment of malaria should be stressed. One of the antimalarials that has been highlighted for its effectiveness is halofantrine. This drug has been used for treatment of human malaria since 1984, and to date clinical trials have involved about 3.4 million patients in more than 30 countries. We report the clinical courses of 14 imported malaria cases that were treated with halofantrine.

The 14 cases consisted of 12 Japanese (9 males and 3 females), 1 Pakistani (male) and 1 Israeli (male), aged from 22 to 53. And of these 14 patients, 7 individuals were infected with *P. falciparum*, 4 with *P. vivax*, 1 with *P. ovale*, 1 with *P. falciparum*+*P. vivax*, and 1 with *P. falciparum*+ *P. malariae*. They were treated with total dosage of 6 tablets of Halfan™ 3 times at 6-hours intervals, with the informed consent of all the patients. One falciparum malaria patient was not successfully treated with Halfan™, whose parasitemia and fever were not cleared. Another falciparum malaria patient

apparently well treated with Halfan™ showed recrudescence of *P.f.* on the 23rd day after the treatment. Other 11 patients recovered from their fever in 42.1 hours (mean) and cleared parasitemia in 52.9 hours (mean), without manifesting severe subjective symptoms of side effects. However, 3 patients who were monitored by electrocardiography on and after Halfan™ administration showed prolongation of QT intervals, one of the cardiac effects of halofantrine.

Halofantrine is an antimalarial drug which is reported to be effective against all species of *Plasmodium*, very well tolerated, and have a simple dosage regimen. It belongs to a class of compounds, the phenanthrene-methanols, which do not share chemical structure with any other antimalarials, and is therefore particularly effective in the treatment of drug-resistant falciparum malaria. However, since 1993, clinical studies have revealed cardiac effects of antimalaria treatment with halofantrine including sudden deaths after the treatment. Finally WHO announced a "drug alert" for halofantrine (Weekly Epidemiological Record, 68, 269-270, 1993), in which described some conditions to be abided by. Further careful studies on individual Japanese patients to confirm its efficacy or tolerance should be required, before halofantrine is generally used in Japan.

A—32

HEAT LOSS RESPONSES OF PIKA (*OCHOTONA CURZONIAE*) WITH THE HIGH ALTITUDE ADAPTATION

NOBU OHWATARI, MUNENORI SHIMAZU, ZONG -WEI LUO AND MITSUO KOSAKA
Department of Environmental Physiology, Institute of Tropical Medicine, Nagasaki University

Pika is classified in *Pika species, Ochtona* genus, *Ochotonidae* family, *Lagomorpha* order. Pika is one of the smaller species in rabbit and is sigular to high altitude adaptation. Pikas (*Ochotona curzoniae*) were trapped on a highland at 3200 m above sea level in Qinghai sheng in China in summer 1994. In this place, the monthly temperatures average ranged from 20.6°C to 23.6°C in summer and −6.7°C to −3.3°C in winter. In the present study, we made experiments to clarify the heat loss of Pika from the insulation of skin and hair with wild Pikas in China.

The body weight and length of 17 Pikas are 195.95± 8.99 g and 19.73±0.24 cm (mean±SE). The skin thickness and hair density are 0.77±0.08 mm and 8.77±0.70 mg/cm² for the chest, 0.79±0.07 mm and 5.80±0.34 mg/cm² for the abdomen, 1.08±0.07 mm and 22.43±1.67 mg/cm² for the back, and 1.11±0.07 mm and 33.97±2.37 mg/cm² for the waist. The back and waist are significantly thick skins and heavy hair compared with those of chest and abdomen. These results suggest that the thermal insulations of back and waist are better than those of chest and abdomen. On the hair types, one is short and soft fur and the others are long and hard hairs of two or three types. It is considered that the fur mainly contribute to thermoregulation due to its thermal insulation. The colors of fur and under half of hairs are dark, the upper half of hairs are light brown. The dark color characteristically absorbs infrared rays and ultraviolet rays compared with light color. Pikas are exposed to cold and strong ultraviolet rays on the highland. The absorption of infrared rays is useful for a solar heat gain, and the absorption of ultraviolet rays at a level of hairs and fur is also useful for a protecting skin.

For Pikas lying on their belly is a behavioral heat loss response, because the heat insulations of chest and abdomen skin were inferior to back and waist. Furthermore the dark color of under hairs of Pika (*Ochotona curzoniae*) is considered to be characteristic of a typical high altitude adaptation in the environment under cold and strong ultraviolet rays.

A—33

BASIC STUDIES ON THE MONGOLIAN GERBIL AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION (15) SERUM ISOENZYME

MASUMI SHIMIZU[1], KAZUHIRO SHICHINOHE[1], KAZUMI IIDA[2], HARUHIRO YOSHIDA[3]
KATSUYA HAGA[4], TETSUO IEDOKORO[4], KOICHIRO FUJITA[4]
Department of Laboratory Animal Science[1] and The Research Institute of Vaccine Therapy for Tumors and Infectious Diseases[2]
Nippon Medical School, Laboratory of Physiology, Senshu University Law School[3]
and Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University[4]

The Mongolian gerbil (*Meriones unguiculatus*) has been well known as an experimental model for filarial infection. Since the coat color mutants of gerbils appeared in 1985 in our laboratory, we established their colonies and conducted the comparative study on response to the filarial infection. We found the coat color differences in reaction to *Brugia pahangi* infection, but physiological genetic variance was not detected until now. In the present study, lactate dehydrogenase and alkaline phosphatase isoenzymes of the gerbil were examined using electrophoretic technique to ascertain genetic polymorphism of those isoenzymes in the coat color mutants of gerbils and were compared with those of the mouse, rat, and guinea pig. Five isoenzymes of lactate dehydrogenase (LDH) were detected in the gerbil and LDH_2 and LDH_5 were equally dominant. Two bands of alkaline phosphatase (ALP) were distinguished in sera treated with neuraminidase in the gerbil and the relative activity of the cathodic fraction was greater than that of the mouse and rat. Genetic polymorphism was not found among the coat color variants of the Mongolian gerbil. Comparative study on LDH and ALP revealed distinct interspecific differences in the rate of the electrophoretic migration of the respective isozymes among the mouse, rat, guinea pig, and the Mongolian gerbil.

A—34

DEVELOPMENT OF MONGOLIAN GERBIL-*BRUGIA PAHANGI* ANIMAL MODEL AS A DISEASE MODEL OF DIETHYLCARBAMAZINE (DEC)-FEVER IN THE PATIENTS WITH FILARIASIS

TAKAAKI MATSUMOTO[1], CALVOPINA MANUEL[2, 4], ZONG-WEI LUO[2], YASUNORI FUJIMAKI[3]
MITSUO KOSAKA[2] AND YOSHIKI AOKI[3]
Department of Thermal Adaptation[1], Department of Environmental Physiology[2]
and Department of Parasitology[3], Institute of Tropical Medicine, Nagasaki University
and Department of Tropical Medicine and Clinical Investigations
Hospital Vozandes, Quito, Ecuador[4]

Diethylcarbamazine (DEC) has been the first choice for the lymphatic filariasis, however, the adverse reactions such as fever, nausea, abdominal pain, dizziness and lethargy, restricted the efficiency of mass chemotherapy of DEC because of the low compliance of the drug. The mechanism of DEC fever in the patients with filariasis is unknown, and the effective means to suppress fever are not established. In this study, Mongolian gerbil-*Brugia pahangi* animal model was developed as a disease model of DEC-filaria fever.

Eight male Mongolian gerbils (*Meriones unguiculatus*) infected with *Brugia pahangi* and 8 un-infected (control) were used. Animals were housed in individual plastic cages in a room maintained at 25°C and 60% rh with a 12h:12h light-dark cycle, with light on at 0600 h. Tap water and rodent chow were provided and libitum. Body temperature and locomotive activity were measured using a biotelemetry system (Dataquest IV, Data Science, USA). The animal was intraperitoneally implanted with a battery-operated transmitter (TA10TA-F20) under ketamine hydrochloride anesthesia, 7-14 days before experimentation began. Output of

the transmitters was monitored by a mounted antenna placed under each animal's cage and fed into a peripheral processor (BCM100) connected to a personal computer. The animal was intraperitoneally injected with DEC (200 mg/kg) at 1130 h.

In filaria-infected gerbils, the body temperature started to rise within 1 hour and reached the maximum rise of 0.62°C 4 hours after DEC injection and persisted to be elevated for 16 hours. The fever, rise in the body temperature was accompanied with a decreased locomotive activity. In contrast, no significant changes in the body temperature or locomotive activity were induced by DEC in control gerbils. Fever index (area under the temperature curve) in filaria-infected and control gerbils were 10.17 ± 2.23 °C×hour and -2.13 ± 3.71 °C× hour, respectively. There was a significant difference between both groups. Repeated administration of DEC showed a tendency of decline in the fever.

In conclusion, it is suggested that Mongolian gerbil-*Brugia pahangi* animal model is the good disease model of the fever induced by DEC in the patients with filariasis. The mechanisms of DEC fever in the filariasis patients will be revealed using this model.

A—35

MARALIA INFECTION IN CENTRAL AFRICAN REPUBLIC

KENJI SHIMODA[1], MORIYASU TSUJI[2] AND KIMITO UCHIYAMA[3]
Division of Medical Technology, Kawasaki College of Allied Health Professions[1]
Department of Tropical Diseases and Parasitology, Kyorin University[2]
and Department of Parasitology, Shinshu University[3]

Parasitic Disease Control Program in Central African Republic started in 1975,and, subsequent to two years' preliminary study, we have been engaged there since 1977 in the treatment of parasitic diseases and the examination of feces, blood and skin of local inhabitants. In this communication we will present the results of 18 years' survey on malaria infection of the region.

The study area consists of five sites: the first (Kella-Sergent village) is in a savanna plain about 1,000m alt. and about 470 km northwest from the capital Bangui; a second (N'dongue yo-yo village) is about 20 km west from a city Bouar, and has a clinical center managed by a Catholic mission from France; a third (Gbaya-baya village) is about 4 km from Bouar; a fourth (Banza village) is a solitary village in a dense forest about 105km west from Bangui, and the fifth is Bangui where the outpatients of National Hospital and Ouango clinical center serve as the subjects.

The percent frequency of plasmodium infected inhabitants ranges from 15 to 86% among the study sites; it is considerably high for all the sites. The percent frequency of malaria infection varies annually, but on average it is the highest in the savanna (Kella-Sergent vil.) which is followed by the dense forest (Banza vil.), while the lowest frequency is found in the capital. Namely, the percent frequency of malaria infection varies depending on sites. The percent infection also varies considerably by month; the percent frequency of malaria infection, and infections with *P. falciparm* and *P. malariae* tend to be higher in dry season than in wet season, though the difference is not specified.

No case was found which was affected with tertian malaria, and indeed we could observe only one case infected with *P. ovale*, one of the causative organisms of tertian malaria. The majority of plasmodium carriers are infected with *P. falciparum* and *P. malariae*. For all the plasmodium carriers, infections with *P. falciparum* are more frequent than those with *P. malariae*, and a considerable portion of them are infected with both species.

A survey on vectors indicates that great swarms of mosquitoes develop in the rainy season around local villages, and the growing sites include pits resulting from the collection of soil by villagers, deserted petrol cans, and small containers thrown off. Mosquitoes including *Anopheles* are also found in hotel rooms in city areas.

A—36

TRYPANOSOMA CRUZI INFECTION KILLS PEOPLE MAINLY IN THEIR WORKING AGE; AN EPIDEMIOLOGICAL STUDY OF CHAGAS DISEASE IN A RURAL AREA OF GUATEMALA

MASAAKI SHIMADA[1], EDMUNDO VELASQUES G[2], KOICHI YAMASHITA[3], ISAO MORIMOTO[4]
KAZUKI OGATA[5] AND ISAO TADA[6]
Dept. of Parasitology and Tropical Public Health, University of Occupational and Environmental Health (UOEH)[1]
University of San Carlos, Guatemala[2], Matsubase Hosp.[3]
1st Dept. of Int. Med., UOEH[4], JICA[5], Dept. of Parasit., Kyushu Univ[6]

This study is an epidemiological survey of 1084 inhabitants in a rural area of Guatemala to determine the prevalence of seropositivity to *Trypanosoma cruzi* as well as the association with chagasic cardiomyopathy. Results of the survey revealed that Chagas disease is highly endemic in this area. Seventy seven (7.1%) of the inhabitants examined by both an indirect hemagglutination test and a fluorescent antibody test were seropositive to *T. cruzi*. Analysis of electrocardiogram (ECG) revealed a significantly high frequency of ventricular conduction defects and arrhythmias among the seropositives. Ventricular conduction defects were observed in 18.2% of the seropositives and 1.7% of the seronegatives, and arrhythmias were 15.6% and 2.7%, respectively. In seropositive individuals, the most common alteration of the ventricular conduction defects was right bundle branch black with or without fascicular block and ventricular premature contraction was most common in arrhythmias. In spite of a strong correlation of ECG abnormalities with seropositivity to *T. cruzi*, the frequency of ECG abnormalities was extremely low as compared to that of seropositives in the age group of 40–59 years. The results may suggest that, in the study area, chagasic cardiomyopathy is one of the important causes of the death of inhabitants particularly in their working age, 40–59 years of age.

A—37

THE EPIDEMIOLOGY OF SNAKE BITE IN NORTHEASTERN ECUADOR

CALVOPINA MANUEL[1,2], GUEVARA ANGEL[1] AND GUDERIAN RONALD[1]
Department of Tropical Medicine and Clinical Investigations, Hospital Vozandes
Quito, Ecuador. South America[1]
Department of Environmental Physiology, Institute of Tropical Medicine,
Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852, Japan[2]

A retrospective study concerning venomous snakebite in the Hospital Francisco de Orellana, located at Amazon rainforests of northeastern Ecuador, South America, was carried out. The study showed that during the last 13 years, 300 cases were recorded, with a prevalence of 23 cases per year. 203 (67.7%) of the affected individuals were male and 97 (32.3%) females. Aged between 2 to 63 years old, with over 50% of bites occurring between 10–29 years. The vast majority of bites occurred on the lower extremities (74.6%). The clinical pattern of envenoming included pain and swelling in 93.1%; one hundred and forty seven patients (49.0%) showed clinical evidence of bleeding at the time of admission suggesting related species of snake as responsible for bites. Only 35(11.6)% of envenomed patients could give a clear description to identify the snake aggressor, *Bothrops spp.* was identified in 31 (90%) cases. Death was reported in two cases (0.7%) as a consequence of severe hemorrhage and hypovolemic shock. Most of the complications observed might be reduced by either earlier treatment with antivenom or earlier referral to hospital.

Key words: venomous snakes, snakebite, epidemiology, Ecuador.

A—38

ANALYSIS OF HELMINTH INFECTION AMONG CHILDREN IN MAHAXAY AREA, LAO P.D.R.

KIYOSHI MAKIYA[1], JUN KOBAYASHI[2], BOUAKHAM VANNACHONE[3]
SOMPHAO BOUNSANA[4] AND YOSHIYA SATO[2]
Department of Parasitology & Tropical Public Health, University of Occupational & Environmental Health[1]
Department of Parasitology, University of Ryukyus[2], Institute of Malariology, Parasitology & Entomology, Lao P.D.R.[3]
and Khammouane Provincial Hospital, Lao P.D.R.[4]

Parasitological survey is going on in Khammouane Province, Lao P.D.R. as a part of Japan/WHO/Laos Primary Health Care Project by the aid of Japan International Cooperation Agency (JICA). A helminthological survey was focussed on children 15 years old or younger in 2 small villages, Nathandong (D) and Nathanthong (T), Mahaxay District, in February-March period in 1995, with the purpose of mass treatment of liver fluke, *Opisthorchis viverrini* (*OV*) . Eggs of the liver fluke (*OV*) were detected from 12 of 66 children (18.2%) in D-village and 12 of 49 (24.5%) children in T-village, respectively using the combined technique of sedimentation and filtration. Egg density (EPG) of *OV* varied widely between 5.0 and 346.7 among the children in D-village and 3.3 and 1600.0 in T-village with the aggregation parameter of negative binomial, $k=0.3792$ and 0.07127, respectively. This means that observed distribution of the fluke is highly aggregated among the children and that much more aggregation was observed in T- than in D-village based on high worm clumping estimated by inverse k value. This is due to the fact that only 2 heavily infected children excreted as many eggs as 90% of all the 12 children infected in T-village while 2 children harbored 45% of the total of 12 in D-village. This tendency of higher worm aggregation in T-village was also observed for *Ascaris lumbricoides* (*AS*) and *Trichuris trichiura* (*TR*) infections, indicating that fewer children expelled more eggs of these worms among the all in T-village.

Most children were infected concomitantly with 2 or 3 worm species. Significant correlation was neither observed between the age of children and worm egg density nor between egg density of each worm species. Mass treatment was carried out by using anthelminthic (Praziquantel) and the evaluation is scheduled to be done based on the post-treatment stool examination.

The similarity of egg density pattern of the 3 worms was analyzed using cluster analysis and the children of the 2 villages were classified into 7 different groups (*TR, OV, AS, OV-TR, AS-TR, AS-OV, AS-TR-OV* (3 Spp) types). Among 6 methods of cluster analysis, Ward's method fitted best for classifying the egg density patterns.

A—39

DRANCUNCULIASIS (GUNIEA WORM DISEASE)

MASAMICHI AIKAWA
Professor, Institute of Medical Sciences, Tokai University, Isehara, Kanagawa 259-11, Japan

Drancunculiasis (guinea worm disease) is caused by the parasite, *Dracunculas medinensis*; people acquire the disease by drinking water contaminated with the parasite. As recontly as the late 1980s, it was estimated that about 120 million Africans and 20 million Asiatics were at risk for guinea worm infection. A campaign begun in 1989 by Global 2000, and WHO and UNICEF has dramatically decreased the number of infected persons. The ultimate goal of this campagn is to entirely eradicate this disease. In July, 1995, as a part of a JICA investigation team on guinea worm disease, we went to Niger and Ghana, West Africa, to study the guinea worm disease situnation. In 1989, there were 179,465 patients in Ghana. In contrast, there were in 1994 only 8,432 such patients (a 95% reduction) after the eradication campaign had been implemented. As of March, 1995, there still were 1.517 patients. In 1993, there were 25,346 patients in Niger: in 1994, the mumber had de-

creased to 16,562 (26.7% reduction). It should be noted that if the erabication program is halted then the disesae will increase in frequency as has been seen in the northern region of Ghana. The target date for the global eradication of guinea worm disease has been set for the end of 1995. Unless education and eradication programs remain in continuous force, it is obvious that the disease will contiune sporadically to reappear. Although the eradication campaign for guniea worm disease has been a spectacular success, this campaign must be continued to eventually totally conquer and wipe out this scourge.

A—40

STUDY ON SCHISTOSOMA SINENSIUM IN NORTHWEST THAILAND

MASANORI KAWANAKA, HIROMU SUGIYAMA[1], NAOHIRO TAGUCHI[2]
SHIIGEHISA HABE[3], HIROHISA HIRAI[4], MIZUKI HIRATA[5]
E.S. UPATHAM[6], TAKESHI AGATSUMA[7]
Dept. Parasitol. National Institute of Health[1], Kouchi Univ.[2], Fukuoka Univ.[3],
Kyoto Univ.[4], Kurume Univ.[5], Mahidol Univ.[6], Obihiro Univ[7]

In Southeast Asia, there are three species of human schistosomes; *S. japonicum, S. mekongi* and *S. malayensis* and five species of animal schistosomes: *S. spindale, S. nasale, S. indicum, S. incognitum* and *S. sinensium*. The three human schistosomes are transmitted by the prosobranch snalils Pomatiopsidae, while these species of animal schistosomes except for *S. sinensium* are transmitted by the pulmonate snails Planorbidae or Lymnaeidae. In the case of *S. sinensium*, it is intriguing of all; the cercariae develop in Tricula which is placed in the family Pomatiopsidae, the intermediate host of human schistosomes in Asia, and the egg possess a lateral spine much more resembles the eggs of *S. mansoni* than that of *S. japonicum*. As interesting possibility is that some character-states shered with both *S. japonicum* and *S. mansoni* are homologous, indicating that *S. sinensium* is derived from a common ancestor to both groups.

On December 1994, a survey on *S. sinensium* was carried out in Fang Distinct, Chang-mai Province, North Thailand, where *Tricula bollingi* snails which harbour cercariae of *S. sinensium* inhabitats. Nine field rats were trapped and examined for the presence of the schistosomes. The stools were found to be positive for *S. sinensium* eggs from three rats. After all nine adult worm pairs of *S. sinensium* were recovered. The worms were examined morphologically and are using as the materials for investigating in molecular genetics.

A—41

PRESENCE OF *SCHISTOSOMA HAEMATOBIUM* EGGS IN VENOUS BLOOD EXAMINED FOR *WUCHERERIA BANCROFTI* MICROFILARIAE

YASUNORI FUJIMAKI[1], C.N. WAMAE[2], YOSHIKI AOKI[1]
Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[1]
Kenya Medical Research Institute[2]

In Kenya, schistosomiasis *heamatobium* is endemic along the Indian Ocean Coast as well as bancroftian filariasis. The present paper reports the finding of 5 cases of *Schistosoma haematobium* (Sh) eggs accidentally detected in venous blood examined for *Wuchereria bancrofti* microfilariae.

Case 1, a 50-years-old man, was found in 1989 by the survey done in Mwachinga, Kwale district. Seventeen Sh eggs were detected on the nucleopore membrane through which 1 ml of night blood taken from the patient was filtered. The number of Sh eggs in urine collected in 1 hour was 302. Cases 2 and 3, 17 and 15 years-old girls, were found in 1991 from Malindi, Kilifi district. A 60 μl sample of finger-prick blood was taken

from each patient and one Sh egg was detected on the filter used for each, after examination of the filter used for dehemoglobinization of blood films. From the latter case 45 Sh eggs were recovered on a membrane used to filter 1 ml night venous blood. The size of 34 out of them were compared with that of 20 Sh eggs in urine collected from another person living in the same area as the patient. There was no significant difference between them. One egg was found in the urine of Case 3 collected in 2 hours. Cases 4 and 5, 17 and 13 years-old girls, were found in 1994 from Muhaka, Kwale district. Two Sh eggs were detected in 1 ml of venous blood from these two subjects. The former was found to have 38 Sh eggs/10 ml urine and the latter 32/10 ml urine.

As far as we know, there are no reported cases of Sh eggs detected in venous blood. Nucleopore membrane filtration technique is the most sensitive tools currently available for the detection of microfilariae. This can be the reason why Sh eggs were detected in venous blood. As the number of membrane-filter screenings for *W. bancrofti* increases in regions where *S. haematobium* exists, the present finding may become much more common.

A—42

AN IMPORTED CASE OF A JAPANESE PATIENT INFECTED WITH *SCHISTOSOMA MANSONI*

OHNISHI KENJI AND MURATA MISAKO
Department of Infectious Diseases, Tokyo Metropolitan Bokutoh General Hospital

A 25-years-old Japanese male patient with schistosomiasis mansoni, infected in Ethiopia, is presented. His blood examination revealed 1,660/mm^3 of eosinophilis (WBC 8,300/mm^3, Eo 20%) and he was diagnosed as having schistosomiasis mansoni by finding *Schistosoma mansoni* eggs in his stool. He was treated with 20mg/kg of praziquantel orally and same dose 4 hours later. A good parasitological therapeutic result was observed, but he complained of abdominal pain, nausea, watery diarrhea and fever after taking the drug. Those symptoms were thought to be side effects of praziquantel, but it is possible that those symptoms may have originated from the degeneration of worms in venules. Schistosomiasis mansoni is not indigenous in Japan, but Japanese doctors should be aware of when they encounter an eosinophilic patient that has returned from or been in a tropical area.

A—43

ULTRASONOGRAPHIC STUDY OF LIVER FIBROSIS IN THE PATIENTS WITH SCHISTOSOME ESOPHAGEAL VARICES IN NORTHEAST BRAZIL

MASANOBU TANABE[1], SEIKI TATENO[2], FERNANDO CORDEIRO[3] AND TSUTOMU TAKEUCHI[1]
Department of Tropical Med. & Parasitol[1], Keio University, International Medical Center of Japan[2]
and Depertment of Gastroenterology, UFPE Hospital PE-Brazil[3]

To clarify the pathogenic mechanism involved in the formation of periportal fibrosis and esophageal varices in chronic schistosomiasis mansoni and identify a most reliable marker for clinical diagnosis of this disease, endoscopic and ultrasonographic examinations were conducted on 76 patients with esophageal varices due to chronic *Schistosoma mansoni* infection in UFPE Hospital, Recife, PE, Brazil. Endoscopic examination of upper digestive tracts demonstrated blue- and white-colored varices in 89.2% and 6.2% of 65 subjects, respectively. Seventeen (29.3%) of 59 subjects were negative for red-color sign, whereas 41 showed red-color sign; 4 red wale marking, 2 cherry-red spot, and 35 hematocystic spot. Form 1, 2 and 3 of the varices were found in 25.4%, 44.4% and 30.2% of 63 subjects, respectively. Enlargements of left liver lobe and spleen were observed

by ultrasonography in 10.7% and 37.7% of these subjects respectively and a definite thickening of the intrahepatic portal vein radicles was detected in all subjects. Dilatation of main portal vein (92.1%), splenic vein (50.9%), left gastric vein (13.2%) and umbilical part of left portal vein (13.2%) were also found in these subjects. 64.4% and 19.2% of 76 subjects revealed an abnormally high value of serum total bile acid and alkaline phosphatase activity, respectively. There was no correlation between the degree of varix and any datum on ultrasonography or biochemical serum analysis, whereas diameters of portal vein system showed a good positive correlation with the degree of periportal fibrosis or spleen size.

Although these findings do not provide a useful information concerning to the pathogenic mechanism involved in the formation of periportal fibrosis, these observations suggest that periportal fibrosis seems to be a most reliable and sensitive marker for clinical diagnosis of chronic schistosomiasis mansoni as reported previously, and also that periportal fibrosis seems to be major causative factor in the formation of esophageal varices in this disease.

A—44

A CASE OF A 1-YEAR-OLD INFANT SUSPECTED OF CYSTICERCOSIS WITH MULTIPLE SPACE OCCUPYING LESIONS IN BRAINSTEM

KENICHI HIBINO, MIKA NISHI, MASAYA KUBOTA, AKIRA OKA
YASUHIDE NAKAMURA, YOICHI SAKAKIHARA MASAYOSHI YANAGISAWA
Department of Pediatics, the University of Tokyo

We reported a case of a 1-year-old infant who has showed fluctuating cranial nerve symptoms, probably attributable to the space occupying lesions due to cysticercosis.

The patient was brought up in a residental quarter and has never played in a sandbox, and never traveled abroad. His parents and grandparents also have never traveled abroad.

At 6 months of age, he showed left exotropia and left ptosis, which recovered spontaneously in a week. He presented mild left facial nerve palsy at 8 months, which recovered within several weeks with prednisolone. At 1-year well-baby examination, left internal strabismus was noticed, and he was refered to our hospital for the evaluation of possible space occupying lesions in brainstem.

On admission, left gaze palsy, left ptosis, left internal strabismus, left facial nerve palsy, and accentuated deep tendon reflex were found. There were, however, no cerebeller and extrapyramidal signs.

The laboratory findings of the blood and the CSF were normal: Serum ELISA revealed negative for cysticercosis, other parasites, toxoplasma, and aspergillus. No tumor cells were found in the CSF.

MRI showed five round cystic space occupying lesions (diameter: about 1cm.) in brainstem with Gd-ringed enhancement. The lesions were very similar to the cysts of neurocysticercosis reported in the literatures.

By treatment with praziquantel 50mg/kg/day for 14 days and steroid hormone for 14 days, cranial nerve symptoms diminished, but MRI was unchanged. 2 months aftar the end of the treatment, cranial nerve symptoms reappeared. Therefore, we used albendazol 20mg/kg/day for 15 days with steroid hormone, which ameliorated the symptoms.

As for the diagnosis of the patient, neurocysticercosis was mostly suspected. Although the locations of the cysts were atypical as cysticercosis,previous case report shows cystic lesions exclusively in brainstem instead of on parenchymal areas where most cysts are located. The similarity of MRI findings and the course of symptoms also support our diagnosis.

The route of infection is still unknown, but there is a report of neurocysticercosis occuring in 2 patients who had never traveled to endemic areas.

Neurocysticercosis is very common in East Asia and Mid-South America, but domestic cases especially of infants are very rare in Japan. Neurocysticercosis should be considered in the differential diagnosis for space occupying lesions of unknown origin, because imported cases of parasitic infections have increased nowadays.

A—45

PRESENT STATE OF SERODIAGNOSIS IN ECHINOCOCCOSIS AND CYSTICERCOSIS

AKIRA ITO
Department of Parasitology, Gifu University School of Medicine

Alveolar echinococcosis (AE), caused by *Echinococcus multilocularis*, and cysticercosis, caused by *Taenia solium*, are the most serious zoonotic metacestode infections in humans. As the clinical manifestations are highly variable, it is not easy to differentiate each other. The most promising means for diagnosis other than clinical manifestations plus US and/or CT image is serological work.

In the present work, we tried to evaluate several different antigen candidates, which have been reported to be species specific, directly by immunoblot analysis. Crude antigens of *E. multilocularis* were prepared from protoscoleces (Em-Ps), those of *E. granulosus* from cyst fluid of sheep (CF-Eg), those of *T. solium* from cyst fluid with or without cyst wall (CF-Ts). Glycoproteins of *T. solium* (GPs) was prepared using Lectin from Culinaris Sepharose 4B (Sigma). These all antigens were used for SDS-PAGE and transblotted onto PVDF using pre-cast gels (TEFCO). Sera from AE, CE (cystic echinococcosis due to *E. granulosus*), cysticercosis, schistosomiasis, paragonimiasis, clonorchiasis, sparganosis, hepato carcinoma, sarcoidosis etc.

Em18 from Em-PS was highly specific to AE, Em-Ps and CF-Eg shared antigen detectable with monoclonal antibody against Em16. GP was highly specific to cysticercosis. CF-Ts contained low molecular antigen (Ts-8) other than GP. It is expected to be species specific and useful for serodiagnosis of cysticercosis. It remains to be solved if Ts-8 is previously undescribed new candidate or not.

The most reliable means for serodiagnosis of AE, CE and cysticercosis are briefly reviewed. Serodiagnosis of AE is based on ELISA, either $Em2^{plus}$ (Gottstein *et al.*, 1993) or PP-Em18/16 (Ito *et al.*, 1996) with immunoblot analysis for detection of antibody against Em18. Serodiagnosis of CE remains to be improved. Serodiagnosis of cysticercosis is based on immunoblot analysis for detection of antibody against GPs or Ts-8.

This work was supported by a Grant-in-Aid for Scientific Research (International Scientific Research Program, Joint Research) from the Ministry of Education, Science and Culture, Japan. The main collaborators are Prof. Liu (China), Prof. Cho (Korea), Prof. Flisser (Mexico), Dr. Schantz (USA), Dr. Lightowlers (Australia), Prof. Craig (UK), Prof. Vuitton (France), Prof. Gottstein (Switzerland) and Japanese colleagues (Prof. Ito Y, Drs. Horii T, Okamoto M, Itoh M, Yamashita T, Nakao M and Mrs. Osawa Y and Ishiguro T).

A—46

THERAPEUTIC EFFECTS OF COMBINATION OF DIETHYLCARBAMAZINE AND SODIUM BICARBONATE ON BANCROFTIAN FILARIASIS

YASUNORI FUJIMAKI[1], YOSHINORI MITSUI[1], S.M. NJENGA[2] AND YOSHIKI AOKI[1]
Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[1], Kenya Medical Research Institute[2]

The parasitological and clinical observations were attempted to examine the effect of a combination of diethylcarbamazine (DEC) with sodium bicarbonate ($NaHCO_3$) on the microfilarial (mf) density of the patients with *Wuchereria bancrofti* infection in Kenya. The mf carriers received DEC at 6 mg/kg or 3 mg/kg with or without $NaHCO_3$ at 75mg/kg body weight. The patients treated by a combination of drugs showed alkaine urine for 4 hours after treatment, while control group showed acidic urine. Although no significant difference in the mean values of all pharmacokinetic parameters between DEC(6 mg/kg) plus $NaHCO_3$-treated and control group (DEC 6 mg/kg), the general tendancy was that in patients receiving $NaHCO_3$ first-

order elimination rate constant (Ke) was decreased, serum elimination of half-life and area under the serum concentration-time curve values were increased. There was significant difference between the mean values of first-order absorption rate constant, Ke and time to peak concentration for the patients receiving DEC at 3 mg/kg plus $NaHCO_3$ and those for the control group receiving DEC at 6 mg/kg alone. There was no difference in frequency and severity of side reactions between $NaHCO_3$-treated and control group. Altough the cure rate was same among the groups, the percent reduction of mf density of the patients recieving DEC plus $NaHCO_3$ was significantly greater than those of patients receiving DEC alone. A combination of DEC with $NaHCO_3$ will be of practical value in a single dose of DEC for the mass treatment of bancroftian filariasis.

A—47

PREPARATION FOR SMALL VESICLES OF *ECHINOCOCCUS MULTILOCULARIS* AND COLORIMETRIC QUANTITATION OF THE VIABILITY OF GERMISAL CELLS

TAMOTSU KANAZAWA[1], HIROKO ASAHI[1] AND KEIZI MOCHIDA[2]
Department of Parasitology[1] and Department of Veterinary Science[2], National Institute of Health

A method of preparation for the small vesicles from *Echinococcus multilocularis* metacestodes and a method of colorimetric quantitation of the viability of germinal cells observed in the vesicles were reported. Percoll step gradient was used to prepare the vesicles. They could be found at 16% and 28% of the Percoll interfaces. The vesicles freshly recovered from metacestodes took up MTT and were stained blue. On the contrary, heat-killed or formalin-fixed vesicles did not absorb MTT. Albendazole sulfoxide suppressed the absorption in a dose-dependent manner. Furthermore, the vesicles which absorbed MTT could infect gerbils with great efficiency, but those not taking up MTT had poor infectivity. It is concluded that theses techniques are of practical value for the preparation of vesicles and the assessment of the viability of germinal cells.

A—48

ANGIOSTRONGYLUS CANTONENSIS INFECTION IN IL-5 TRANSGENIC MICE WITH SPECIAL REFERENCE TO THEIR RESISTANCE TO INFECTION AND ADHESION MOLECULE EXPRESSION IN EOSINOPHILS

HIROKO SUGAYA[1], MIKIKO AOKI[1], KENTARO YOSHIMURA[1]
AND KIYOSHI TAKATSU[2]
Department of Parasitology, Akita University School of Medicine[1] and
Department of Immunology, Institute of Medical Science, University of Tokyo[2]

Non-permissive hosts (e.g., humans, mice and guinea pigs) infected with *Angiostrongylus cantonensis* provoke marked peripheral and cerebrospinal fluid (CSF) eosinophilia, whereas developing worms in those hosts die sooner or later in their brains. Contrarily, no blood and CSF eosinophilia can be noted in *A. cantonensis* infected nude mice or mice treated with anti-mouse IL-5 monoclonal antibody, where pulmonary migration of the parasite frequently occurs (Yoshimura *et al.*, 1982; Sugaya & Yoshimura, 1988; Sasaki *et al.*, 1993); these findings strongly suggest that murine Th2 cells produce IL-5 cytokine whereby induced CSF eosinophilia is probably involved in the killing of intracranial worms. Our study was thus conducted to determine whether IL-5 transgenic mice (13-19-weeks-old female mice) infected with 30 third-stage larvae of *A. cantonensis* can develop a stronger worm killing activity than control C3H/HeN (9-weeks-old female mice). The kinetics of adhesion molecule (VLA-4, ICAM-1, CD11b and CD11a) expressions in eosinophils was also

monitored in *A. cantonensis* infected IL-5 transgenic mice.

Blood eosinophil level of uninfected IL-5 transgenic mice was 137 times higher than that of C3H/HeN mice. Blood eosinophil level decreased in IL-5 transgenic mice following *A. cantonensis* infection but CSF eosinophils significantly increased during days 10 to 25 postinfection and the peak CSF eosinophil level at day 20 was approximately fivefold higher than that of C3H/HeN mice. Intracranial worm recovery at days 5 and 10 postinfection was significantly lower in IL-5 transgenic mice than in C3H/HeN mice, and subsequent worm reduction was also distinct in IL-5 transgenic mice, which yielded a significantly lower worm recovery at day 25 postinfection than C3H/HeN mice.

Flow cytometry was performed to assess adhesion molecule expression in blood and CSF eosinophils. The assay revealed that VLA-4 expression in CSF eosinophils was significantly reduced at days 14 and 20 and then recovered. To the contrary, CD11b (Mac-1) expression was found increased only at day 14 postinfection. Meanwhile, blood eosinophils showed no appreciable changes in the expression of any of adhesion molecules. The density of eosinophils was assayed by discontinuous Percoll gradient centrifugation. Blood eosinophils from uninfected IL-5 transgenic mice and infected mice at days 14 and 20 showed a normal density (1.0725 g/ml), whereas 50% and 98% of CSF eosinophils at days 14 and 20 respectively, showed a lower density (less than 1.070 g/ml). In short, CSF eosinophils could be associated with worm killing in the brains of mice infected with *A. cantonensis*.

A—49

EOSINOPHIL-SPECIFIC EXPRESSION OF gp91-*Phox*

ATSUSHI KUMATORI[1], SHOICHI SUZUKI[1], FUTOSHI KURIBAYASHI[1]
MICHIO NAKAMURA[1], YOSHIRO TSUJI[2]
Department of Biochemistry, Institute of Tropical Medicine[1] and
Department of Pediatrics, Faculty of Medicine[2], Nagasaki University, Japan

Phagocytes stimulated with bacteria or parasites generate superoxide anion by integrating NADPH oxidase system. The system contains at least four essential components, namely a small and a large subunits of cytochrome b_{558} (p22-*phox* and gp91-*phox*) and two cytosolic components (p47-*phox* and p67-*phox*). A deficiency of either one of the components results in chronic granulomatous disease (CGD) with severe recurrent infections. All kinds of phagocytes of x-linked CGD patients have been reported to be deficient in gp91-*phox* as expected from the fact that gp91-*phox* gene is single per haploid and lies on X chromosome. Eosinophils of an X-linked male patient, however, fully expressed surface cytochrome b_{558}, indicating that gp91-*phox* is apparently normal. His eosinophils generated superoxide anion to a normal extent and definitely expressed the large subunit of cytochrome b_{558} (gp91-*phox*). His mononuclear leukocytes contained a trace amount of gp91-*phox* mRNA with an apparently normal size. All the coding sequences and a putative poly (A) signal sequence of his gp91-*phox* gene were normal. We found a point mutation in the 5'-flanking sequence of his gp91-*phox* gene. Gel shift assay with intact and mutated sequences indicated that monocytes and neutrophils but not eosinophils contain nuclear protein(s) which specifically bind to the intact sequence but not to mutated one. These results indicate that eosinophils have a specific mechanism for the expression of gp91-*phox* gene, and monocytes and neutrophils have a common mechanism for the expression of the gene. Elucidation of these mechanisms may give a clue to control superoxide generating activity individually in eosinophils and other phagocytes under conditions such as parasite infections and allergy.

A—50

PCR PRIMER TO DETECT GENOMIC DNA OF *TOXOCARA CANIS* AND *T. CATI*

YUZO TAKAHASHI, WU ZHILIANG, ISAO NAGANO, XU DONG
Department of Parasitology, Gifu University School of Medicine

T. canis and *T. cati* infect dogs and cats, and may cause visceral larva migrans to non-definite hosts, humans. To assess enviromental contamination by the eggs of *Toxocara*, a highly reliable and simple method is needed. In this paper we report PCR primer for *T. canis* and *T. cati*.

Genomic DNA was prepared from several adult worms obtained from different dogs and cats in Hyogo Prefecture in Japan. In brief the worms were extensively washed, homogenized, and digested with Pronase K. The DNA was precipitated after a phenol/chloroform extraction, and suspended in a buffer. Using such DNA as a template, AP-PCR was carried out and DNA fragrents were isolated from a dense bane. The DNA fragment was sequenced and primers were synthesized as follows: 5'-AGCCGAAAGTGTATCAAGGA- 3', 5'-TGATGTGCTTGCCGCTGTTA-3'. Regular PCR was carried out with a profile of 92 C for 30 sec 51 C for 30 sec and 72 C for 60 sec repeating 30 cycles. The tested DNA samples included *T. canis, T. cati, Dirofilaria immitin, Taenia saginata, Trichinella spiralis*. A single band with 293 bp was obtained in the samples of *T. canis* or *T. cati*, but not in those of the other parasites. Thus the primer we deleloped seems to be promising to detect DNA of *T. canis* and *T. cati*.

ACKNOWLEDGEMENT The authors thank Dr. Uga in Kobe University for providing samples.

A—51

CHARACTERIZATION OF A GENE FOR THE 34KDA EGGSHELL PRECURSOR PROTEIN OF *SCHISTOSOMA JAPONICUM*

SUGIYAMA, H[1]., KAWANAKA, M[1]., KAMEOKA, Y.[2],
NAKAMURA, M[3]., AND ASANO, A[3].
Department of Parasitology[1] and Division of Genetic Resources[2],
Natioal Institute of Health, Institute for Protein Reseach, Osaka University[3]

We have cloned the cDNA encoding part of the 34 kDa eggshll protein of *Schistosoma japonicum* (Jpn. J. Trop. Med. Hyg., 22, 57, 1994) This clone, designated Sj -23, containing a 230-bp insert, showed no sequence homology at both the nucletide and predicted protein levels to the eggshell gene, p14 (Bobek *et al.*, 1986) and p48 (Chen *et al.*, 1992) from *S. mansoni*. Sj-23 was, however, shown to be homologous to the eggshell genes of *Fasciola hepatica* (Zurita *et al.*, 1987; Rice-Ficht *et al.*, 1992).

A longer cDNA clone, SjP34, containing a 985-bp insert, was newly isolated by using Sj-23 as a probe. The SjP34 was compared with the *F. hapatica* eggshell gene, F3. Sequence homologies were 45% and 22% at the nucletide and polypeptide levels, respectively. The eggshell protein deduced from SjP34 was rich in tyrosine (11.1%) and glycine (12.1%). This observation agrees well with the composition of the eggshell protein deduced from F3. RT-PCR analysis revealed that SjP34 was expressed solely in mature female *S. japonicum*. The homologue of this gene was detected in the *S. mansoni* genome using PCR. Further studies are in progress to isolate genomic clones and to investigate the regulation mechanism of SjP34.

A—52

MAPPING OF A NEWLY ISOLATED EGGSHELL PRECURSOR PROTEIN GENE ON *SCHISTOSOMA JAPONICUM* CHROMOSOMES

YOSUKE KAMEOKA[1], HIROMU SUGIYAMA[2], KEIKO KATO[2]
AND MASANORI KAWANAKA[2]
Division of Genetic Resources[1] and Department of Parasitology[2]
Natitnal Institute of Health

The eggshell genes, p14 (Bobek *et al.*, 1986) and p48 (Chen *et al.*, 1992), have been isolated from *Schistosoma mansoni*. It is known that the two genes hybridized to different portions of chromosome 2 (Hirai *et al.*, 1993).

Sj-23 has been obtained as a cDNA clone which encodes a part of the 34 kDa eggshell protein of *Schistosoma japonicum* (Jpn. J. Trop. Med. Hyg., 22, 57, 1994). The nucleotide sequence of this cDNA dose not show any homology to p14 and p48 from *Schistosoma mansosi*. Recently, another cDNA clone, SjP34, containing 985-bp insert, was obtained by using Sj-23. To identify the locus of this clone SjP34, FISH (fluorescence *in situ* hydridization) was carried out. The metaphase chromosomes of *S. japonicum* were prepared from intramolluscan stages following techniques by Hirai *et al.* 1993). SjP34 was labeled by PCR to obtain biotinilated products of length 300 to 800bp using appropriate primers. Hybridized probes were detected by using anti-biotin rabbit IgG, biotin conjugated goat anti-rabbit IgG, avidin-alkali phosphatase and HNPP (3-hydroxy-N-2'-biphenyl-2-naphthalenecarboxyaminde phosphate). 25 male and 25 female metaphase chromosomes were observed under a florescence microscope, and analyzed the locatin of the signals. The hybridiza tion signals were mainely observed on the terminal of q-arm of chromosome 1. The accumulation rate of the signals was 30%. FISH was also carried out on the metaphase chromosomes of *S. mansoni*. The signals were observed on the same position and accumulation rate 30%.

These findings show that the locus of SjP34 is independent from that at of p14 or p48 and the homologue of SjP34 in conserved in the same position of *S. mansoni* Chromosome.

A—53

A MOLECULAR PHYLOGENY OF THE GENUS *PARAGONIMUS* FROM A VIEWPOINT OF ITS 2 REGION OF THE NUCLEAR RIBOSOMAL RNA GENES

TAKESHI AGATSUMA[1] AND DAVID BLAIR[2]
Department of Bioresource Chemistry, Obihiro University of Agriculture and Veterinary Medicine[1]
and Department of Zoology, James Cook University[2]

In this study, internal transcribed spacer 2 (ITS2) of the nuclear ribosomal RNA genes was examined among species of the genus *Paragonimus* and an attempt to construct a phylogenetic tree was made. *Paragonimus* species used in this study were *P. westermani* (Pw) from Japan(j), Taiwan(t), Philippines(f) and Malaysia(m), *P. ohiral* (Po) from Kisosaki, *P. iloktsuenensis* (Pi) from Amami, *P. miyazakii* (Pm) from Nankoku, *P. heterotremus* (Ph), *P. kelikotti* (Pk), *P. mexicana* (Pmex) and *P.* sp(Psp(co)) from Colombia.

PCR amplification was carried out using the conditions which have been reported previously (Agatsuma et al., 1994). After PCR amplification, the products were purified using HPLC and microcon, and applied for sequencing reaction using Dye Terminator method (ABI). For computor analysis, Neighbor Joining (NJ) and Parsimony methods were used to construct phylogenetic trees. As a result, 1) Po and Pi were grouped as a single cluster, and were found to be closely related to Pm, 2) the Thai Ph was clustered with Pm, 3) the American Pk was clustered with Pmex, 3) Psp(co) were found related to Pmex, 4) geographically distant

populations of Pw were clustered as a separate group, and within the Pw group, two clusters, Pw(f)/Pw(m) and Pw(t)/Pw(J) were found, 5) with the Parasimony method, Po/Pm cluster was grouped with the American *Paragonimus*, however, the NJ method showed that the Po/Pm cluster was more related to the Asian Pw cluster.

A—54

DIAGNOSIS OF DIARRHEAL CASES IN RWANDAN REFUGEES IN GOMA, FAIRE

HIROSHI SHIOMI[1], JUNICHI OKADA[1], MITSUO TADA[1], AKIHIKO SATAKE[2]
SHIGEHIKO IWATA[1], SEIJI SHIBAHARA[1], TAKUMI ASAO[1], HIDEAKI ICHINOSEKI[2]
Ground Self Defence Force, Air Self Defence Force

Japanese Task Force to Relief Rwandan Refugees detached to Goma (Zaire) through September to December 1994 was requested by the UNHCR's Goma office to tmansfer technology to AMI-KIVU laboratory of North Kivu State of Zaire. UNHCR especially wished us to train the local laboratory technicians on the bacterial culture methods in order to get detailed informations on the surveillance of bacterial diarrhea including cholera and shigellosis in these areas. We not only trained local staffs of the laboratory, but also took samples from several refugee camps in coordination with NGOs, transported these samples to AMI-KIVU and examined them as follows.

1) Transport specimens within 3 hours.
2) Direct observation on microscope to check parasites.
3) Cuture on TCBS agar and on SS agar.
4) If large yellow colony is aparent on TCBS agar, pick up and examine it with Gram-staining. If Gram negative rods are detected, check it on API-System^R to characterize it biochemially.
5) If lucent colorless colony is aparent on SS agar, pick up it and reculture it on SS agar. If similar lucent colony is detcted, examine it on Enterotube^R to characterize it biochemially.
6) If pathogen is diagnosed as V. cholerae or Shigella on the above procedure, confirm them with antiserums.
7) Examine antibiogram on Muller-Hinton agar.

These procedure were modified in the training of local staffs because API-System and Enterotube are expensive and cold chain is not available in Goma. We collected as many as 475 difrrheal stool specimens and examined them. On detection of shigella we found lucent colorless colonies on SS agar in 95 cases, in which 15 were diagnosed as Shigella, 14 cases were examined on the anti-serums (S. dysenteriae: 2, S. flexneri: 8, S. nonnei: 3, S. boydii: 1, respectively). On detection of V. cholerae we found yellow colonies in 33 cases, in which Gram negative rods ware found in 2 cases. Only 1 case was diagnosed as V. choleare on API-System, which agglutinated with Ogawa-antiserum. These pathogens were sensitive to nalidixic acid and new quinolones, but resistant to tetracycline, ampiciline and chloramphenicol.

Detected Shigellas and V. cholerae were not so many as expedted. In refugee camps there were still considerable number of diarrhea cases. We observed a lot of enteric parasites such as Entamaeba histolytica, Giardia lamblia and Trichomonas intestinalis. These persisting diarrheal cases would be explaind mainly by these prevailing parasites.

A—55

IMMUNOLOGICAL BACKGROUND IN INFANTS WITH DIARRHEA IN GHANA

KIYOSU TANIGUCHI[1], TAKASHI NAKANO[1], HITOSHI KAMIYA[1], MINORU SAKURAI[2]
TOSHIYA KAMIYA[3], TORU RIKIMARU[3], F.K. NKRUMAH[3]
Department of Pediatics, Mie National Hospital[1], Department of Pediatrics, Mie University School of Medicine[2]
Noguchi Memorial Institute for Medical Research, University of Ghana[3]

Persistent diarrheal diseases have become one of the most serious medical problems in developing countries, but few studies have been conducted to determine the risk factors. The present study will compare the nutritional and immunological background in children with persistent diarrhea and with acute diarrhea.

All children with diarrhea, who are brought to the ORS clinic at the outpatient department of Princess Marie Louise (PML) Children's Hospital in Accra, the capital of Ghana, are target population. After informed-consent, the child was registered and mother was interviewed about personal information and episode of diarrhea. The child was examined physically and anthropometrically. 5 ml of blood sample was taken for biochemical, serum immunoglobulin measurement and lymphocyte surface marker analysis. The mother was encouraged to record the change in diarrhea epiodes and to report back every week for follow up. During follow-up visit, the cases whose diarrhea stopped within 2 weeks after onset were classified into acute group; those diarrhea lasting more than 2 weeks as persistent group. Laboratory data at initial visit were examined in comparison between these two groups. All diarrhea cases were treated by the pediatrician in charge of PML hospital by their usual manner.

Eighty-seven cases with diarrhea were recruited and investigated. They were 50 males and 37 females with average age of 16.3 months.

In 25 cases of these, diarrhea persisted more than 2 weeks. Using the Waterlow Classification, it seemed that there were no significant relationship between anthropometrical status and duration of diarrhea. The biochemical analysis shows that rapid turn over protein has no significant difference between acute and persistent diarrhea group. Serum immunoglobulin subclass and complement level had no impact on persistence of diarrhea. In lymphocyte surface marker analysis, $CD3^+$ percentage was significantly higher in persistent group than that in acute group. Persistent group has higher percentage of $CD8^+$ and lower value of CD4/CD8 ratio. Lymphocyte activation assay using anti-CD3 antibody showed CD25 expression in $CD4^+$ cells are impaired in persistent diarrhea group.

Abovementioned results suggests that impairment of T-cell activation related to the occurrence of persistent diarrhea.

A—56

STRUCTURE AND FUNCTION OF RECOMBINANT PIG RECEPTOR (STaR) FOR A *ESCHERICHIA COLI* HEAT-STABLE ENTEROTOXIN (STa) IV: CHARACTERIZATION OF CARBOXY-TERMINAL REGION OF HEAT-STABLE ENTEROTOXIN RECEPTOR

AKIHIRO WADA AND TOSHIYA HIRAYAMA
Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University

Heat-stable enterotoxin receptor (STaR/GC-C), an endogenous receptor protein for guanylin, is a member of membrane-associated guanylyl cyclase (GC) family. STaR has a unique prolonged carboxy-terminal tail following the GC catalytic domain, which has 63 or 62 extra amino acids compared with the other member of the mebrane-associated GCs, natriuretic peptide receptor (NPR-A/GC-A or NPR-B/GC-B). In comparison with the recently found membrane-associated GCs in the eye and olfactory neurons, retGC, GC-D, GC-E, or GC-F, the carboxy-terminal tail of STaR is also prolonged by about 30 amino acids. In the prolonged

carboxy-terminal tail of STaR, three separable regions such as Pro rich region (PXPP), uncharged polar region (Gln or Ser rich region), basic region (Arg or Lys rich region), and the characteristic STaR-tail region exist in that order. However, it is not known whether this carboxy-terminal tail is essential for STa induced-GC activation or not. Four deletion mutants of STaR lacking the carboxy-terminal tail were transiently expressed on 293T cells, and then their GC activities were determined by the presence or absense of STa. The STaR mutant lacking a characteristic STaR-tail region was activated by STa whereas other STaR mutants lost their inherent property of STa-induced GC activation. Accordingly, the basic region of STaR in carboxy-terminal tail play, at least in part, an important role for STa-induced GC activation.

A—57

EPIDEMIOLOGY OF INFLUENZA VIRUS INFECTIONS AMONG CHILDREN WITH ACUTE RESPIRATORY INFECTIONS (ARI) IN ZAMBIA.

KATSUMI MIZUTA[1], HITOSHI OSHITANI[3], MWILA EVANS MPABALWANI[2]
NAKANDU LUO[2], HIROSHI SUZUKI[3], YOSHIO NUMAZAKI[3]
Department of Pediatrics, National Miyagi Hospital[1],
Virology Laboratory, University Teaching Hospital[1], Lusaka, Zambia[2],
Virus Research Center, Sendai National Hospital[3]

A community based study was designed to define the etiology and epidemiology of viral acute respiratory infections (ARI) in children. Throat swab specimens were collected from children under 5 years old with ARI, attending at health centers in Lusaka, Zambia. A total of 4,000 specimens were collected between June 1993 and August 1995. Specimens were inoculated into microplate including HEF, HEp-2, Vero, and MDCK cell lines. Influenza viruses were isolated on MDCK cell line and isolates were identified with Hemagglutination-Inhibition (HI) test. In 1993, 54 influenza A/H3N2 were isolated between June and November. In 1994, 36 influenza B were isolated between May and July. In 1995, one influenza A/H3N2 was isolated in January and then the same type of influenza virus was isolated between June and August. The isolation rate was highest, 15.1% in August 1993, 16.0% in June 1994, and 23.8% in July 1995 in each season. These results suggested that influenza virus infections are one of the most important pathogens of ARI in children in the cool dry season (June-August) in Zambia. Although there are few reports from African region, our results also suggested that the same type of influenza virus had been circulating in the Southern African region between 1993 and 1994 season, according to the Weekly Epidemiological Record of World Health Organization (WHO).

A—58

UTILITY OF POLYMERASE CHAIN REACTION (PCR) METHOD FOR RAPID LABORATORY DIAGNOSIS OF ENTEROVIRUS INFECTION

T.N. DOHLE, SHIN ISOMURA
Sanjay Gandhi Post-Graduate Institute for Medical Sciences. Lucknow, UP. INDIA

OBJECTIVE To test utility of PCR as a rapid method to identify the isolated enteroviruses.
METHODS The oligometric region of great homology among enteroviruses were identified and designated as potential primer OL 68-1 (−), EV-D3 (+) and probe PV-1, for PCR to identify the enterovirus strains isolated from patients with cell culture methods.
RESULTS All the clinical materials were collected from children in Lucknow, India, during a period of 1993-94.

Out of 100 strains of enteroviruses which were isolated by HeLa, VERO and RD-18S cells from children with diarrhea, polio or other illnesses, 30 strains were identified as poliovirus, 20 as Coxackie A and B virus, and 50 as ECHO virus, respectively.
CONCLUSION PCR has potential clinical applicability in the laboratory diagnosis of enterovirus infection with the rapidity and reliability.
ACKNOWLEDGMENTS This work was done by collaboration with Prof. Ayyagari and Dr. Mishra (SGPGI), Dr. Sakae and Dr. Yamashita (Aichi Prefectural Institute of Public Health).

A—59

CHARACTERIZATION OF POLIOVIRUSES ISOLATED FROM WEST PAKISTANI CHILDREN

AYAKO HASEGAWA , OSAMU NISHIO, SAKAE INOUYE
HIROSHI USHIJIMA, SHIN ISOMURA
National Institute of Health, National Institute of Public Health, The University of Tokyo, Nagoya University

Poliomyelitis eradication is now one of the important problems in WHO. We isolated polioviruses from stool specimens collected in West Pakistani children during 1990 to 1993 and characterized whether these isolates originated from vaccine strains.

Stool specimens were collected from 14 poliomyelitis, 6 diarrheal patients, and 6 healthy children. Viruses were isolated in MA104 cells and isolated viruses were identified using antisera to polioviruses. The genomic variability of poliovirus was examined by analyzing the restriction fragment length polymorphism of a reverse-transcribed genomic fragment amplified by the polymerase chain reaction (PCR-RFLP). Three restriction enzymes, HaeIII, DdeI and HpaII, were used.

Of 26 specimens 23 enteroviruses were isolated and 9 were identified as poliovirus; one was type 1, 5 were type 2, 1 was type 3, and 2 were a mixture of type 1 and 2. Two strains of type 1 had different RFLP patterns from that of Sabin 1. Other one did not produce a genomic fragment by PCR. One type 2 strain was identical to Sabin strain, and other 6 strains showed different patterns from Sabin 2. Those 6 strains were divided into two groups according to the RFLP patterns. Probably, two kinds of wild poliovirus type 2 were cocirculating in this area during 1990 to 1993. One type 3 isolate was found to be derived from Sabin strain.

We then tried to determine the nucleotide sequences of amplified genomic fragments of the wild poliovirus type 2 isolates. The results were in agreement with that of the FRLP profiles; 5 strains were divided into two groups.

These results indicate that wild polioviruses are circulating in West Pakistan. For the elimination of wild polioviruses it is necessary to get the information in wild poliovirus prevalence, and laboratory diagnosis can provide valuable information.

A—60

MEASLES INFECTION IN HIV SEROPOSITIVE CHILDREN IN ZAMBIA

HITOSHI OSHITANI[1], HIROSHI SUZUKI[1], MWILA MPABALWANI[2], KATSUMI MIZUTA[3]
YOSHIO NUMAZAKI[1]
Virus Research Center, Sendai National Hospital[1],
Virology Laboratory,University Teaching Hospital, Zambia[2]
Miyagi National Hospital[3]

Clinical presentations and outcome of measles infection were compared between human immunodeficiency virus type 1 (HIV-1) seropositive and seronegative hospitalized children. A total of 664 children with clinical diagnosis of measles were recruited at the University Teaching Hospital, Lusaka, Zambia between

January 1993 and October 1994. Blood samples were collected and tested for HIV antibody using ELISA (Wellcozyme HIV-1, Wellcome, U.K.) and particle agglutination (PA) (Serodia HIV, Fujirebio, Japan). Among those studied, 127 were positive for HIV-1 antiboby, making overall HIV positive rate 19.1 %. The case fatality rate (CFR) was significantly higher in HIV seropositive children than in seronegative (24.4 % vs. 7.6 %). And the CFR decreased by age in HIV seronegative children (12.6 %, 7.6 %, 7.8 % and 1.3 % in those aged under 9 months, 9-17 months, 18-59 months and over 60 months, respectively), on contrary the CFR was high also in older children in seropositive children (12.5 %, 29.4 %, 25.0 % and 17.6 % in those aged under 9 months, 9-17 months, 18-59 months and over 60 months, respectively). Complications such as pneumonia, oral candidiasis and otitis media were more common in HIV seropositive children, however diarrhea was equally seen in both HIV seropositive and seronegative groups. In HIV seronegative children CFR was significantly lower in previously vaccinated children, however in HIV-1 seropositive group CFR was even higher in vaccinated children. We conclude that measles infection is more severe in HIV seropositive children, and that the current vaccination is not adequate in immunosuppressive children. Therefore the new strategy should be established to protect HIV infected children against measles infection.

A—61

COMPARATIVE ANALYSIS OF ANTI-TUBERCULOUS CHEMOTHERAPY BETWEEN 2REHZ/6RH AND 2SEH/10EH FOR HIV-SEROPOSITIVE PATIENTS WITH PULMONARY TUBERCULOSIS IN UGANDA

SATOSHI MITARAI[1], KAZUNORI OISHI[1], HIROSHI YAMASHITA[1], TSUYOSHI NAGATAKE[1]
ALPHONSE OKWERA[2] AND KEIZO MATSUMOTO[1]
Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University[1]
Old Mulago Hospital, Makerere University School of Medicine, Uganda[2]

Sixty-five patients were enrolled into a treatment trial for patients with pulmonary tuberculosis and HIV infection. They were randomized into two different groups. The one group involving thirty four patients was treated with Regimen 1: rifampicin, ethambutol, isoniazide and pyrazinamide for first two months and rifampicin and isoniazide for additional six months. Another group involving thirty was treated with Regimen 2: streptomycin, ethambutol and isoniazide for first two months and isoniazide and ethambutol for additional ten months. The two groups were not statistically different about the age, CD4 and CD8 cell number in peripheral blood at the enrollment (Age; 30.0 ± 6.9 and 31.0 ± 5.7, CD4 cell number; $156\pm110/\mu l$ and $134\pm76/\mu l$, CD8 lymphocyte number; $310\pm125/\mu l$ and $322\pm206/\mu l$). The rate of resistance against anti-tuberculosis drugs are 8.0% against isoniazide and 2.3% against streptomycin. No resistant strain was detected against rifampicin and ethambutol in our study so far. The successful treatments of each regimen are 30 of 35 patients (85.7%) in Regimen 1 group and 22 of 30 patients (73.3%) in Regimen 2. No significant difference in the cure rate and safety was observed between the Regimen 1 and 2. The CD4 and CD8 cell number did not changed significantly despite of a successful treatment. Finally, the two regimens are very safe and effective treatment for the pulmonary tuberculosis associated with HIV-seropositive patients. The Regimen 2 is especially recommended on the economic view in developing countries.

A—62

AN EPIDEMIOLOGICAL STUDY ON KAPOSI'S SARCOMA IN WESTERN KENYA FOR 15 YEARS

KAN TORIYAMA, HIDE-AKI ETO, MASAKO KUSUDA AND HIDEYO ITAKURA
Department of Pathology, Institute of Tropical Medicine, Nagasaki University

We analysed an epidemiological transition of Kaposi's sarcoma (KS) in the western part of Kenya, East Africa during the 15-years period between 1980 and 1994. The western part of Kenya accounts for one third of the whole country in area and about one half in population. It is bounded by Sudan and Ethiopia on the north, Uganda on the west and Tanzania on the south and consists of Western, Nyanza and Rift Valley Provinces. KS is classified on historical and epidemiological background as follows, a) Classical form occuring among people in Eastern Europe and in the Mediterranean countries, b) Endemic form peculiar to African natives in equatorial Africa, c) Epidemic form in HIV-infected or AIDS patients, d) Other forms in patients after organ transplantation who have been treated with immunosuppressive therapy. It is thought that AIDS has spread over this area since the middle of the 1980s. When compare the results of epidemiological study on KS before and after that date, 1) The frequency of KS amongst all malignant tumors has increased to 3.8% from 2.6%, 2) The male to female ratio has shifted to 6:1 from 9:1, 3) Two peaks of the age distribution in the early childhood and the middle age group have changed to three peaks, including the adolescent age group, 4) The mean age of the patients has shifted to 36 years from 40 years, 5) The frequency of disseminated and aggressive cases with lymph node involvement among the adult patients has increased, 6) KS is rapidly spreading among the inhabitants in tropical highland and semi-arid area, where very few patients were found before. These findings suggest that African endemic type KS, which has no relation to HIV infection, is being rapidly replaced by AIDS-related KS in the western part of Kenya, East Africa.

A—63

VIROLOGICAL STUDY ON AN IMPORTED DENGUE FEVER CASE

KEN-ICHIRO YAMADA[1], SADAO YABE[1], YOHKO T. ARAI[1], TAISUKE HORIMOTO[1], MIKIO NAKAYAMA[1]
KIICHI YAMAMOTO[1], OSAMU ISHIMOTO[2], SHINICHI OOSHIMA[2], MAKO UCHIDA[2], HAJIME YUASA[2],
TADAHIKO KITANO[1] AND MASATO TASHIRO[1]
Dept. Virology 1, National Institute of Health[1], Seirei Mikatahara Hospital[2]

Dengue fever and dengue haemorrhagic fever (DF/DHF) are now of major public health problems in countries in the west pacific region such as Thailand, Vietnam and Philippines. Dengue (DEN) virus infections do not occur in Japan, but they are occasionally imported by travelers who have visited tropical countries and become infected with the viruses. Sera of patients with fever unknown origin are occasionnally brought to our laboratory for diagnosis of DEN virus infections. The present paper reports a case of imported dengue fever patients. Serum specimens were collected from the patient who had traveled in Thailand and Vietnam during April 14 to May 19, 1995.

To detect and identify the type of DEN virus in the serum specimens, we carried out HI test, detection of the viral genome using the RT-PCR and isolation of virus using mosquito cells (C6/36 and TRA-284-SF). The results were as follows: 1) Serum HI antibody titers against DEN type 2 of serum on days 3, 7 and 10 post onset of the disease were less than 1 : 10, 1 : 80 and 1 : 2,560, respectively. HI titers against Japanese encephalitis virus were 1 : 10, 1 : 80 and 1 : 10,240 respectively, 2) DEN type 1 viral genome was detected by RT-PCR from the serum of day3 (NK-3), 3) The serum (NK-3) was inoculated to two mosquito cell lines, and the cultured fluids were harvested after incubation for 7 days. DEN type 1 viral genome was detected in these fluids by RT-PCR. 4) DEN virus was also isolated in infected mosquito cells. CPE appeared in TRA-284-SF cells infected with the serum (NK-3) after incubation for 6

~7 days, whereas no CPE was observed in infected C6/36 cells. 5) The isolated virus was identified as DEN type 1 by the neutralization test using peroxidase-anti-peroxidase (PAP) staining method.

A—64

SEROEPIDEMIOLOGICAL AND VIROLOGICAL STUDIES ON JAPANESE ENCEPHALITIS IN VIENTIANE, LAO P.D.R.

MIKA SAITO[1], BOUNLAY PHOMMASACK[2], KAZUMI KANERUMA[1], SHAO-PING MA[1], KHAMPE PHILAVONG[3], DUANGDAO SUKALUNG[4], BOUNXOU SOSENGSOURIDETH[2], KHANTHONG[2], BOUAKET PHOTHISACK[2], BOUNMY[2], SAKAE ARAKAKI[1], YOSHIHIRO MAKINO[1], TOSHIHIKO FUKUNAGA[1], SITHAT INSISIENGMAY[5]
Department of Virology, Faculty of Medicine, University of Ryukyus[1]
Vientiane Municipality Health Service[2], Sethathirath Hospital[3]
Mahosot Hospital[4], National Institute of Hygiene and Epidemiology, Lao P.D.R.[5]

Lao P.D.R. is located in Indochina Peninsula, has never reported the epidemiscs of Japanese encephalitis (JE) although neibouring countries, such as Thailand and Vietnam, reported severe epidemics. In order to know the real situation of JE in Lao P.D.R., we conducted epidemiological study in the following way: 1) virus isolation from pig sera and their analyses, 2) serological study on JE-suspected patients and healthy children.

1) Two virus strains (LaVS56 and LaVS145) were isolated from pig sera obtained at slaughter house in Vientiane, using C6/36 mosquito cell line. These virus strains were identified as JE virus (JEV) using JE monoclonal antibodies and RT-PCR. We examined a 240 nucleotide sequence of the Pre-M gene region of the strains and examined their genetic relationships to the other JEV from a variety of geographic areas in Asia. Lao isolates belong to the group of the genotype of northern Thailand and Cambodia based on the classification of Chen WR. *et al.* (1990).

2) A total of 45 cases of viral encephalitis were hospitalized in 1994. Laboratory diagnosis was carried out by neutralization test against dengue virus (type 1, 2, 3 and 4), and JEV (Nakayama, Beijing, LaVS145, LaVS56 and p19 of Chiang Mai strain) and by IgM capture ELISA. IgM specific to JEV were detected in sera from two cases. These cases also showed significant rise in neutralization titers against Nakayama and Beijing, but not against LaVS56, LaVS145 and p19. Neutralization antibody titers against Beijing were 10 to 363 times higher than these against Nakayama.

Other 2 cases showed high IgM specific to dengue virus in acute sera. Therefore these were suspected as dengue encephalopathy.

A total of 268 sera from healthy children under 13 years old were tested for neutralization antibodies against 3 strains of JEV (Beijing, LaVS145 and Nakayama). Thirty-eight sera showed positive for antibody against any one of the strains. These sera appeared to be divided into two groups; one which neutralized LaVS145 strains more than Beijing and Nakayama, and the other which neutralized Beijing and Nakayama more than LaVS145.

A—65

INHIBITORY EFFECT OF SURAMIN ON JAPANESE ENCEPHALITIS VIRUS REPLICATION

KE-SHU XU AND TSUTOMU TAKEGAMI[1, 2]
Division of Tropical Medicine, Medical Research Institute[1]
and Department of Microbiology[2], Kanazawa Medical University

Japanese encephalitis occurs in endemic and epidemic form over a wide area of Asia, at least tens of thousands of cases occur annually in East Asia. Although the vaccine against JE virus (JE¥) is widely

used, we have no antiviral drugs against JEV replication. Suramin has been developed as the antitrypanosomal agents and recently it has been suggested that it has some inhibitory effects on the DNA and RNA polymerase. Here we describe an antiviral activity of suramin on JEV replication.

In the presence of 50 μg/ml suramin, virus yield in human neuroblastoma cell line, IMR32, was reduced 0.1% of control level. JEV growth in human hepatoma cell line, HepG2, was also inhibited, but inhibitory effect of suramin was lower than that in IMR32. The difference of inhibitory effects between host cells suggests that some host factors were involved in the inhibition of JEV growth. By Western blot analysis, it was clarified that expressions of JEV proteins, NS3 and E were markedly reduced by the treatment of suramin at 50-200 μg/ml. Especially the expression of E protein seems to be sensitive against suramin treatment. However, JEV-RNA level in cells treated with suramin was not so different from control level, and *in vitro* JEV-RNA synthesis was also not influenced by the addtion of suramin. These results suggest that suramin inhibits virus replication through the influence to viral protein production.

Proceedings of XXXVII Annual Meeting of Japanese Society of Tropical Medicine

ISSN 0304-2146

JAPANESE JOURNAL OF TROPICAL MEDICINE AND HYGIENE

VOL. 24 NO. 1 March 1996

CONTENTS

(Contents continued on reversed of this cover)

JSTM FOUNDED 1959

Jpn. J. Trop. Med. Hyg.

JAPANESE SOCIETY OF TROPICAL MEDICINE