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EFFICACY OF ANTIBIOTICS AGAINST EXTRACELLULAR AND INTRACELLULAR *BURKHOLDERIA PSEUDOMALLEI*, AND THEIR THERAPEUTIC EFFECTS ON EXPERIMENTAL PEUMONIA IN MICE

HIROSHI FUKUHARA, TOMOHIKO ISHIMINE,
MITSUHIKO FUTENMA AND ATSUSHI SAITO

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Abstract: We examined the effects of 14 antibiotics on the isolated strains of *B. pseudomallei* from melioidosis patients in Thailand. The organisms caused pneumonia and was found to survive in macrophages in mice. Thirteen strains of *B. pseudomallei* isolates showed resistance to avariety of antibiotics such as ampicillin, cefazolin, cefotiam, gentamycin, erthromycin, and clindamycin. Piperacillin, ceftazidime, ceftizoxime, minocycline, ciprofloxacin, levofloxacin and ofloxacin were moderately active antibiotics. Imipenem was the most active antibiotic. Efficacy of imipenem and levofloxacin were also examined against the organism in the macrophage. Imipenem showed the strong MIC, (MIC=0.125 mg/L), but a 4×MIC dose did not inhibit intracellular growth of the organism in mice peritoneal macrophages. In contrast, a 4×MIC of levofloxacin inhibited the intracellular growth although the MIC=1 mg/L. Levofloxacin therapy showed a significantly ($P<0.01$ or $P<0.05$) higher survival rate than imipenem/cilastatin, piperacillin and minocycline against experimental *B. pseudomallei* pneumonia in mice. Imipenem/cilastatin and ceftazidime showed almost the same effectivity against the infection, although imipenem/cilastatin required half the dosage of ceftazidime. Levofloxacin may be the first choice in the treatment of melioidosis caused by *B. pseudomallei*.

Keywords: *Burkholderia pseudomallei*; *Pseudomonas pseudomallei*; Melioidosis; facultative intracellular pathogen; Levofloxacin

INTRODUCTION

Melioidosis is an infectious disease of man and animals caused by the Gram-negative rod species of bacterium *Burkholderia pseudomallei* (formerly *Pseudonomas pseudomallei*) (1). The disease is endemic in tropical countries which locate between latitude 20° north and 20° south such as southeast Asiaand northern Australia (2). However, melioidosis has occasionally occurred elsewhere probably carried by travellers or military personnel from such endemic areas (3). Clinical manifestations of melioidosis are septicemia, pneumonia and visceral abscess (4).

The mortality rate of severe melioidosis has been improved by the introduction of ceftazidime to be treatment since 1983 (5). Recently, Parksachartvuthi et al (1990) reported that *B. pseudomallei* showed is a characteristic feature as facultative intracellular bacterium (6). We have therefore examined the MIC and MBC of 14 antibiotics against isolated *B. pseudomallei* as well as the effects of antibiotics on the intracellular organism in mice macrophage. The therapeutic efficacy of 5 kinds of antibiotics was also examined by eliciting pneumonia with the isolates in mice. We followed guideline measure for biohazard-control.

MATERIALS AND METHODS

Bacterial strains

Thirteen strains of *B. pseudomallei* were kindly provided by Dr. Prasit Tharavichitkul and Dr. Charlie Phornphutkul, Faculty of Medicine, Chiang Mai University, Thailand. These strains were kept in sterile skimmed milk pH 7.4 (Difco Inc, Detroit, USA), at −80°C,

First Department of Internal Medicine, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-01, Japan

and incubated for 48 hr before use in each experiment. All strains had been recently isolated from sputum or blood of clinical pneumonia or sepsis cases in Thailand. *B. pseudomallei* SP-237 was obtained from sputum culture of pneumonia.

Antimicrobial agents

The following antibiotics were used in this experiment: ampicillin (Meiji Pharmaceutical Co. Ltd, Japan), piperacillin (Toyama Chemical Co. Ltd, Japan), cefazolin (Fujisawa Pharmaceutical Co. Ltd), cefotiam (Takeda Chemical Industries, Ltd, Japan), ceftazidime (Glaxo Japan Laboratories Co. Ltd, Japan), ceftizoxime (Fujisawa Chemistry Co. Ltd, Japan), imipenem (Banyu Pharmaceutical Co. Ltd, Japan), gentamicin (Sionogi & Co. Ltd, Japan), minocycline (Lederle Japan Ltd, USA), ciprofloxacin (Bayer Japan Co. Ltd, Japan), ofloxacin (Daiichi Pharmaceutical Co. Ltd, Japan), levofloxacin (Daiichi Pharmaceutical Co. Ltd, Japan), clindmycin (Upjohn Japan Co. Ltd, Japan), and erythromycin (Dainihon Pharmaceutical Co. Ltd, Japan).

MIC and MBC determination

The organisms were grown overnight in CSMHB {cation supplemented Mueller-Hinton Broth (BBL, Cockeysill, USA); Mueller-Hinton broth 1 liter+Mg^{2+} 25mg/L+Ca^{2+} 50mg/L by incubator in room air at 35°C}. The inoculum size was determined by diluting an overnight growth of bacteria and comparing the turbidity with a 0.5 McFarland standard. The bacterial cultures were diluted in 0.9% saline to achieve a final concentration of 1×10^5 CFU/ml. Subsequently these were inoculated in U-bottom microdilution trays along with control organisms and incubated aerobically for 24 hr at 37°C. The concentrations of antibiotic after inoculation ranged from 64 to 0.0625mg/L. The MIC was determined as the lowest dilution of antibiotic concentration that showed no turbidity after overnight incubation in room air at 35°C. The MBC of *B. pseudomallei* SP-237 was defined as the lowest dilution of antibiotic that completely inhibited growth on nutrient agar (BBL, Cockeysvill, USA) after a further overnight incubation in room air at 35°C.

Intracellular antimicrobial activity

The mice were injected intraperitoneally with Roswell Park Memorial Institute(RPMI) medium. The mononuclear cells were adjusted to a concentration of 1×10^6cell/ml, by counting cell in a Neubauer counting chamber after staining with Tripan blue solution. The cell suspension (1 ml) was placed in each well of a 24 well flatbottom tissue culture plate (Nuncon, Denmark) and incubated in the presence of 10mg/L gentamycin for 3 hr in 5% CO_2/air at 37°C. After incubation, the wells were washed five times with sterile warm RPMI medium to remove nonadherent cells. The adherent cells were referred to below as the macrophages. *B. pseudomallei* SP-237 (1×10^7 cfu) and marophages (1×10^6) were incubated in RPMI medium for 1 hr. *B. pseudomallei* SP-237 did not multiply in the medium after the macrophages were washed five times. New media with 0.5 mg/L imipenem (4×MIC), and 4 mg/L levofloxacin (4×MIC) were added to wells and incubated in 5% CO_2/air at 37°C. After 0, 3, 6, 12, 24 hr, the cultured macrophages were harvested in 9 ml of sterile distilled water, then vortexed for 30 sec to lyse the macrophages completely with out killing the bacteria. The bacterial suspensions were diluted appropriately, and 0.1ml was inoculated on nutrient agar to determine the number of viable *B. pseudomallei* SP-237 in each well.

Experimental B. pseudomallei pneumonia in mice model

Six-week-old male Bulb/c mice weighing 18-20g were purchased from Shizuoka Laboratory Animal center (Shizuoka, Japan), fed in a specific pathogenfree (SPF) environment with a 12 hr light-dark cycle and used for the experiments. *B. pseudomallei* SP-237 was used throughout this experiment. Mice were anesthetized subcutaneusly with 0.25 ml of 10% pentobarbital sodium (Dainihon Pharmaceutical Co. Ltd, Japan) and infected by the intratracheal route with 3.2×10^3 cfu per 0.05ml.

Lung stamp specimen from *B. pseudomallei* SP-237 infected mice

The stamped specimens of the dissected lung were obtained at 72 hr after infection from mice receiving levofloxacin therapy or none at all. These specimens were stained with acridine orange and were observed through the fluorescence microscope. Levofloxacin therapy (10mg/kg) was initiated at 24 hr after the inoculation. Levofloxacin was administered orally twice a day for 60 hr. Lungs of controll mice were observed before inoculation.

Treatment schedule

Five antibiotics were chosen with relatively high activity as measured by MIC. The infected mice were injected subcutaneusly (ceftazidime, imipenem/cilastatin, piperacillin, minocycline, or vehicle), or administered orally (levofloxacin) twice a day for 7 days. Each

dose was 200mg/kg(ceftazicime, piperacillin), 100mg/kg(imipenem/cilastatin) or 10mg/kg(levofloxacin, minocycline) starting at 24 hr after infection. Control mice received 0.1ml of physiological saline(vehicle). Each group consisted of ten kinds, n=10. Mortality was recorded daily for 14 days. The statistical significance of data was determined by the generalized Wilcoxon test.

Table 1 Minimum inhibitory concentration (MIC) of 14 antibiotics against 13 isolates of *B. pseudomallei*

Antibiotics	Range	MIC(mg/mL) 50%	90%
Penams			
Ampicillin	8～>64	>64	>64
Piperacillin	0.5～1	1	1
Cephems			
Cefazolin	>64	>64	>64
Cefotiam	64～>64	64	64
Ceftizoxime	0.5～1	1	1
Ceftazidime	0.5～1	1	1
Carbapenem			
Imipenem	0.125	0.125	0.125
Quinolones			
Ofloxacin	2～8	2	4
Ciprofloxacin	1～4	1	2
Levofloxacin	1～4	1	2
Aminoglycoside			
Gentamycin	>64	>64	>64
Tetracyclin			
Mynocycline	0.5～1	1	1
Lincomycin			
Clindamycin	>64	>64	>64
Macrolide			
Erythromycin	>64	>64	>64

n=13

RESULTS

MICs and MBCs

The results of drug susceptibility tests with *B. pseudomallei* are shown in Table 1. Imipenem was the most active antibiotic. Piperacillin, ceftazidime, ceftizoxime, minocycline, ofloxacin, levofloxacin and ciprofloxacin were moderately active antibiotics which had a MIC_{90} of ≦4ug/ml. Ampicillin, cefazolin, cefotiam, gentamycin, clindamycin and erythromyc in showed resistance againstall strains of *B. pseudomallei.*

The MIC and MBC of 13 antibiotics against *B. pseudomallei* SP-237 are shown in Table 2. Imipenem was most strong bactericidal activity. Piperacillin, ceftazidime, ceftizoxime, and levofloxacin were moderately strong bactericidal activity. Minocycline did not

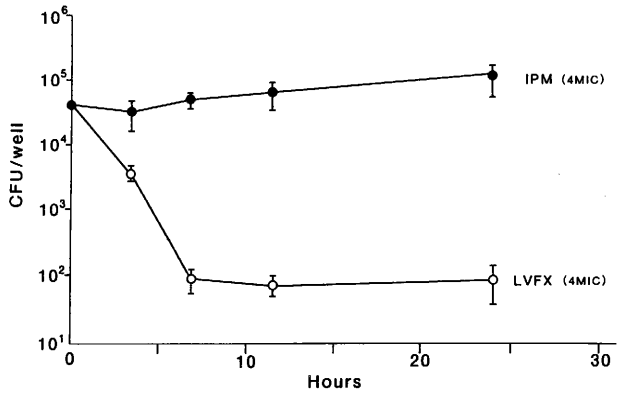

Figure 1 Intracellular growth of *B. pseudomallei* in peritoneal macrophage prepared from mice. Macrophage monolayers were inoculated, incubated for 3 hr and then washed. Either levofloxacin or imipenem was added at 1 hr after inoculation with the bacterium. Each point represents the mean viable count in cfu±S.D. of nutrient agar.

Table 2 Comparision of MIC and MBC of 13 antibiotics against *B. pseudomallei* SP-237

Antibiotics	MIC	MBC(mg/L)	Antibiotics	MIC	MBC(mg/L)
Penams			Quinolones		
Ampicillin	>64	>8	Ofloxacin	2	4
Piperacillin	0.5	1	Levofloxacin	1	2
Cephems			Aminoglycoside		
Cefazolin	>64	>8	Gentamycin	>64	>8
Cefotiam	>64	>8			
Ceftizixime	1	2	Tetracyclin		
Ceftazidime	1	2	Mynocycline	1	8
Carbapenem			Lincomycin		
Imipenem	0.125	0.5	Clindamycin	>64	>8
			Macrolide		
			Erythromycin	>64	>8

(a)

(b)

(c)

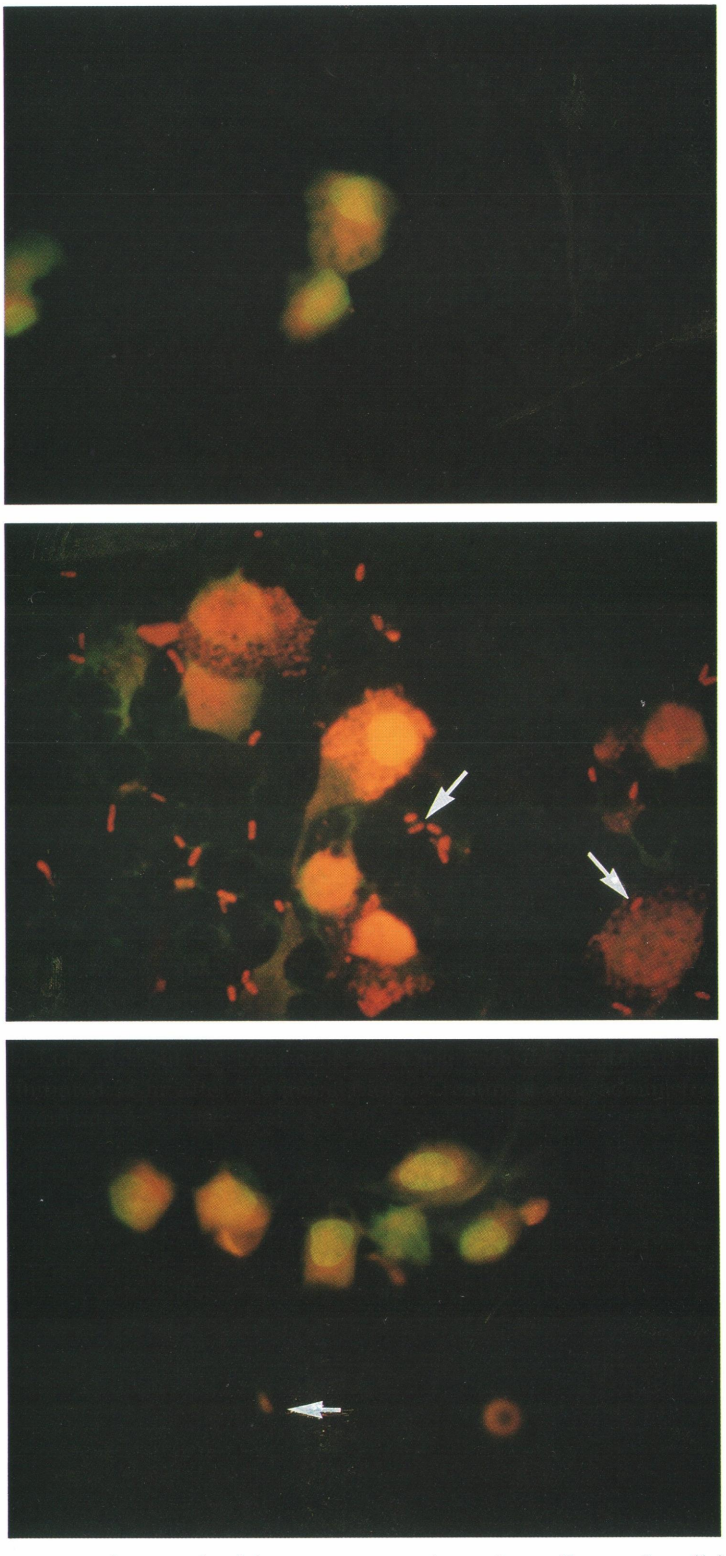

Figure 2 Fluorescence micrograph of lung stamp specimen from *B. pseudomallei* infected mouse by the intratracheal route at 72 hr after the inoculation (3.2×10^3 cfu per 0.05ml). Intracellular and free organisms with acridine orange stain were observed (arrow). (a) Normal lung. (b) Lung without the treatment. (c) Lung with the treatment; levofloxacin (10mg/kg) was initiated 24 hr after the inoculation. Treatment was administrated orally twice a day. Magnification: ×1,000

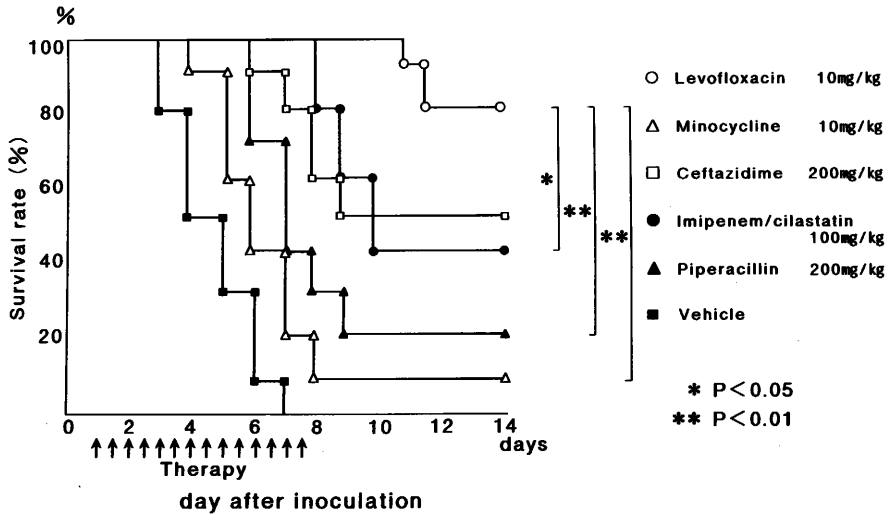

Figure 3 Cumulative survival rate of experimental pneumonia by the intratracheal route with *B. pseudomallei* (3.2×10^3 cfu per 0.05 ml) in mice in treatment and vehicle group. Treatments were given subcutaneusly (ceftazidime, imipenem/cilastatin, piperacillin, minocyclin, or vehicle) or administrered orally (levofloxacin) twice a day for 7 days. Control mice received 0.1 ml of physiological saline (vehicle).

show strong activity with MBC. *B. pseudomallei* SP-237 showed resistance as measured by MIC and MBC against ampicilin, cefazolin, cefotiam, gentamycin, clindamycin and erythromycin.

Intracellular antimicrobial activity against *B. pseudomallei*

The number of bacteria in the macrophages were shown in Figure 1. The imipenem at 0.5mg/L (4×MIC) did not inhibit the intracellular growth of *B. pseudomallei* whereas levofloxacin at 4mg/L (1×MIC) remarkably inhibited.

Existence of *B. pseudomallei* SP-237 in the lung of infected mice with or without therapy

Intracellular and free organisms were strained in lung stamp specimens from mice infected with *B. pseudomallei* SP-237 without therapy with acridine orange and observed by fluorescence microscope. No organisms were observed in the lungs of control mice and one variation of free organis was observed in lungs of the infected mice receiving therapy (Figure 2).

Effects of antibiotics against experimental *B. pseudomallei* pneumonia in mice model

The ability of five antibiotics to affect the survival rate of mice infected with *B. pseudomallei* is shown in Figure 3. All control mice receiving vehicle alone died within 7 days after infection. Incontrast, mice treated with levofloxacin showed an increase in survival (80%). Levofloxacin therapy showed a significantly ($P<0.01$ or $P<0.05$) higher survival rate than minocycline (10%), piperacillin (20%) and imipenem/cilastatin (40%).

DISCUSSION

Melioidosis was first described in Rangoon in 1912 (7). In recent years, melioidosis has been recognized as a common cause of illness and death in northeast Thailand (2). There have been two reports of Japanese melioidosis patients to date (8, 9). Melioidosis is a disease which is associated with high mortality rate, particularly the pneumonia and sepsis forms (2-4).

B. pseudomallei is naturally resistant to many antibiotics (10-12). The mortality rate of severe melioidosis was reduced from 70% to 40% by ceftazidime therapy (13). We expected to find an improved therapy because the survival rate is still low. We therefore examined the activity of 14 antibiotics against *B. pseudomallei in vivo*. Imipenem showed the highest activity, followed by piperacillin, ceftazidime, ceftizoxime, minocycline, levofloxacin, ciprofloxacin and ofloxacin. The MBC of antibiotics were 2-4 fold less than MIC except in the case of minocycline. Minocycline's MBC may be a factor of eight less than its MIC by reason of it being a bacteriostatic drug. Livermore et al (1987) reported *B.*

pseudomallei produced beta-lactamase of the same weakly inducible but other factor (e.g. poor drug penetrationb or low target affinity) may contribute to the beta-lactams resistance of the organism(14). *B. pseudomallei* is a facultative intracellular growth of organism such as the genera *Salmonella, Shigella* and *Listeria*(15, 16, 17). We speculated that *B. pseudomallei* survived and multiplied in phagocytes despite of therapy with effective antibiotic with MIC against the pathogen *in vitro*. Beta-lactams have generally poor penetration into phagocytes(18). Gaja et al(1992) found that the ratios of intracellular/extracellular drug concentration of levofloxacin in human neutrophils was 8.83±1.27(19). Imipenem 0.5mg/L(4×MIC) did not inhibit the intracellular growth of *B. pseudomallei* in macrophages, whereas levofloxacin 4mg/L(4×MIC) could inhibit it. Levofloxacin was significantly more effective than ceftazidime, imipenem/cilastatin, piperacillin and minocycline against *B. pseudomallei* pneumonia in mice. Penetration of levofloxacin into the lung was presumably higher than that of ciprofloxacin, as lung concentrations attained with the related drug ofloxacin after oral administration was 2-3 fold higherthan ciprofloxacin (19). Levoxacin, the (S)-(-)-isomer of ofloxacin, showed generally twice the antibacterial activity of ofloxacin(21). Ofloxacin given orally was more effective than ciprofloxacin against *Salmonella typhimurium* infection in mice despite of ciprofloxacin being twice as effective as ofloxacin *in vitro*(15). As *Salmonella* is a facultative intracellular bacterium, levofloxacin may be able to penetrate in to macrophages more effectively than ciprofloxacin. The ability of *B. pseudomallei* to survive and multiply in macrophages may be supposed to be the cause of the difficulty of treatment of melioidosis and the high rate of relapse.

In summary, levofloxacin showed high intracellular activity *in vitro* and was effective against *B. pseudomallei* pneumonia in mice *in vivo*. Levofloxacin may have a useful clinical role in melioidosis caused by *B. pseudomallei* in humans.

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The authors gratefully thank Mr. I. Nakasone for his technical assistance and Dr. N. Kusano and Dr. F. Higa for their encouragement.

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TRYPANOSOMA EVANSI: UNIQUE CONCAVITIES ON THE SURFACE MEMBRANE OF PARAROSANILINE-INDUCED AKINETOPLASTIC CLONES AS REVEALED BY SCANNING ELECTRON MICROSCOPY

MARY ROSE AGNES SILVA-TAHAT*, AKITOYO ICHINOSE, HARUKI UEMURA AND HIROJI KANBARA

Received December 20, 1994/Accepted February 28, 1995

Abstract: Pararosaniline-induced akinetoplastic clones of *Trypanosoma evansi* which lack DAPI-stainable kDNA network were characterized by scanning electron microscopy. Independent batches of akinetoplastic parasites from two passages in mice were observed to have similarities with parental kinetoplastic strain with respect to shape, form, and pleomorphism. The marked difference in surface topography was noted between the wildtype and the mutant as unique concavities on the cell surface of the latter. These concavities are variable in size, number and extent and may be inheritable. In addition, akinetoplastic cells were found to undergo active longitudinal binary fission and filopodia formation as reported by others. These observations suggest that the kDNA-deficient mutants of *T. evansi* have maintained their basic functions of cell division and infectivity and, therefore, the concavities on their surface are not detrimental to their existence.

Keywords: Scanning electron microscopy, *Trypanosoma evansi,* akinetoplastic form, surface concavity

INTRODUCTION

Trypanosoma evansi is a haemoflagellate which causes a wasting disease called surra (also as desrengadera or murrina in local language) in domestic animals particularly camels, horses and cattle. It has a wide geographical distribution ranging from North Africa, Asia and South America. Although it is most closely related to *Trypanosoma brucei,* it has deviated to a very simplified life cycle without invertebrate-related stages and, hence, morphology, as an adaptation to a mechanical mode of transmission by horseflies and other biting insects (Hoare, 1967, 1972).

However, like all other members of the order Kinetoplastida, *T. evansi* possesses a kinetoplast DNA (kDNA) albeit its reduced size which reflects the lack of respiratory processes in the mitochondrion (Borst and Hoeijmakers, 1979). In lieu of this feature, this trypanosome is susceptible to mutate spontaneously into forms with altered kDNA, the so-called dyskinetoplastic and akinetoplastic forms (Hoare, 1954). Spontaneous mutants account for about 3-6% of a given population (Inoki *et al.*, 1962). These mutants are morphologically similar to the wildtype except for the marked difference revealed by transmission electron microscopy as the replacement of the kDNA network by an electron-dense body referred to as the kinetoplast remnant (Vickerman and Preston, 1976). In addition, although the fundamental functions of the akinetoplastic cells are not much different from the kinetoplastids with respect to viability, infectivity, and ability to proliferate, we have previously observed that the akinetoplastic parasites have a delayed rate of cell division and, therefore, a slower growth (silva-Tahat et al., Res., in press).

In the present paper, we further characterize pararosaniline-induced akinetoplastic clones of *T. evansi* by scanning electron microscopy and describe their morphological features. We show that only the mutants have unique surface concavities which may be inheritable and further demonstrate that they undergo active longitudinal binary fission and filopodia formation indicating that the concavities are not harmful for the parasites.

Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Japan 852
*To whom all correspondence should be addressed.

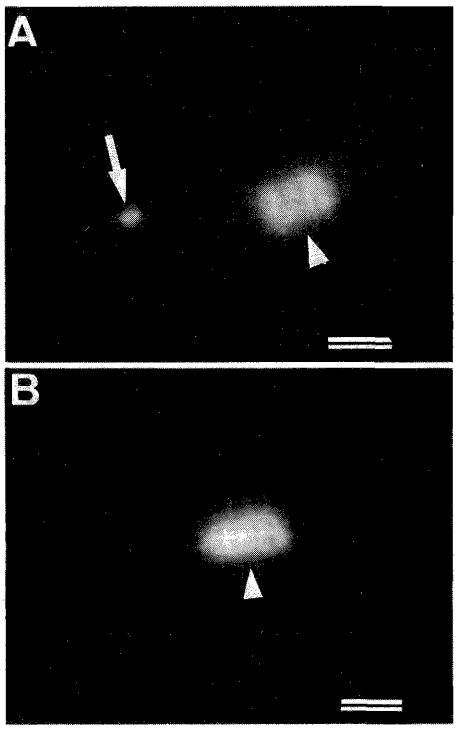

Figure 1 DAPI fluorescence micrographs. (A) Kinetoplastid with 2 fluorescent particles, a large nuclear DNA (arrowhead) and a small kDNA (arrow). (B) An akinetoplastic form with a single large fluorescent body, i.e., the nuclear DNA. Bars, 1 μm.

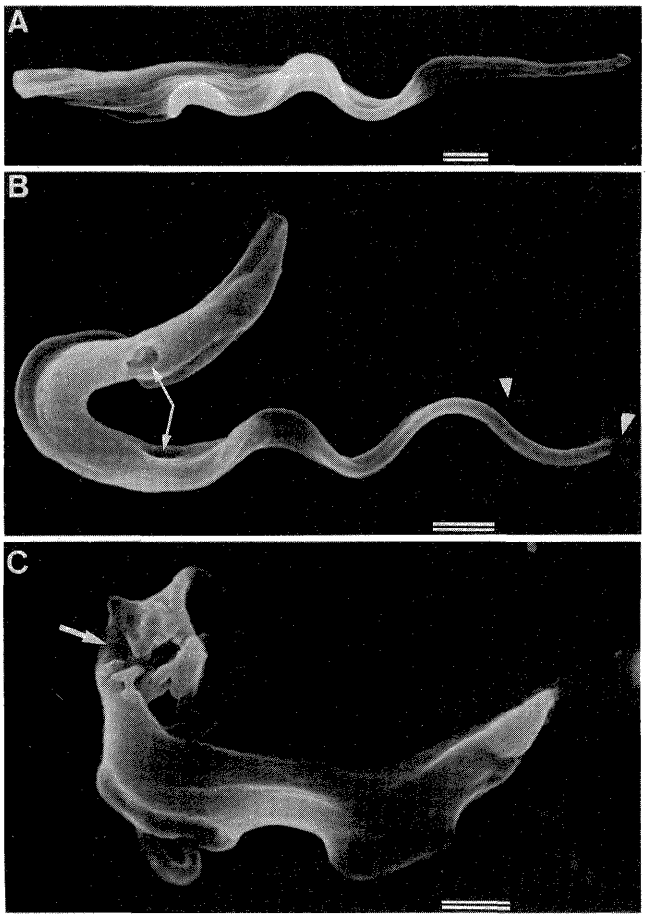

Figure 2 Scanning electron micrographs. (A) A representative photomicrograph of the kinetoplastid. Note typical trypanosome surface topography. (B) Akinetoplastic clone bearing concavity at the lower third of the cell body and a more shallow one at the anterior region (conjoined arrows). Short processes of segmented filopodia (arrowhead) arise from the surface of the free flagellum. (C) A number of deep potential concavities (arrow) gathered together at the anterior portion of the akinetoplastic body. Bars, 1 μm.

MATERIALS AND METHODS

Parasites

A kinetoplastic Tansui strain of *Trypanosoma evansi* isolated from a waterbuffalo was kindly provided by Dr. K. Fujisaki (National Institute of Animal Health, Tsukuba) and was used in the study. Parasites were cultured and maintained by passage in 8-week-old ICR mice.

Induction and cloning of akinetoplastic parasites was accomplished according to Inoki (1960). Briefly, the kinetoplastic strain was induced to become akineto plastic by successive injections of infected mice with 10-20 μg/g of pararosaniline. Akinetoplastidy was assessed by 2,4-diamidino-6-phenylindole (DAPI) staining of tail blood samples following the method of Hajduk (1976). Mutants were eventually cloned by single-cell isolation and propagated in mice. The akinetoplastic nature of the mutants was further confirmed by South ern hybridization analysis using kDNA probes (silva-Tahat et al., in press).

Electron Microscopy

Infected blood sample was collected by cardiac puncture. Parasites were isolated and purified by passage through DE52 anion exchange column. Both kinetoplastic (passage 5, K5) and established akinetoplastic clones (passages 3 and 4, AK3 and AK4, respectively), which have been maintained in the absence of the dye, were suspended and washed in 0.16M phosphate-saline-glucose (PSG) buffer (pH 7.4) consisting of 0.0113M $NaH_2PO4.2H_2O$, 0.0486M Na_2HPO_4, 0.0436M NaCl, and 0.0555M dextrose prior to fixation in 2% gluteraldehyde. Bloodstream trypomastigotes were

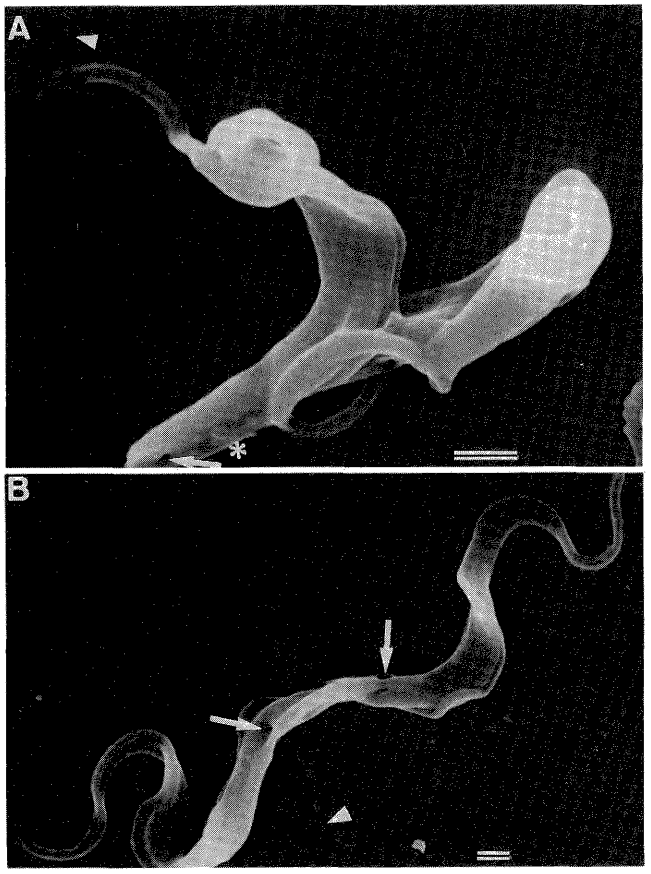

Figure 3 Scanning electron micrographs of akinetoplastic forms during cell division. (A) A trypanosome which bears a small concavity (arrow) at the posterior end is in the process of longitudinal binary fission. One of the daughter cells extends a filopodium (arrowhead) from the flagellum. Vesicular structures (asterisk) on the surface of the posterior cell body may denote primordial filopodia. Both daughter cells exhibit a slight degree of torsion at the anterior regions. (B) Presumably the final stage of cell division whereby daughter trypanosomes are still attached at their posterior broad terminal prior to complete division and separation. Note the presence of concavities (arrow) at the fused broad ends of both trypomastigotes and filopodium (arrowhead) in the same region of the lower trypomastigote. The upper trypanosome is very slightly twisted. Bars, 1 μm.

subsequently prepared for scanning and transmission electron microscopy following standard procedures.

Results and Discussion

Pararosaniline caused the deletion of a DAPI-fluorescent kDNA in *Trypanosoma evansi* (Figure 1). The absence of the kDNA in the akinetoplastic parasites was

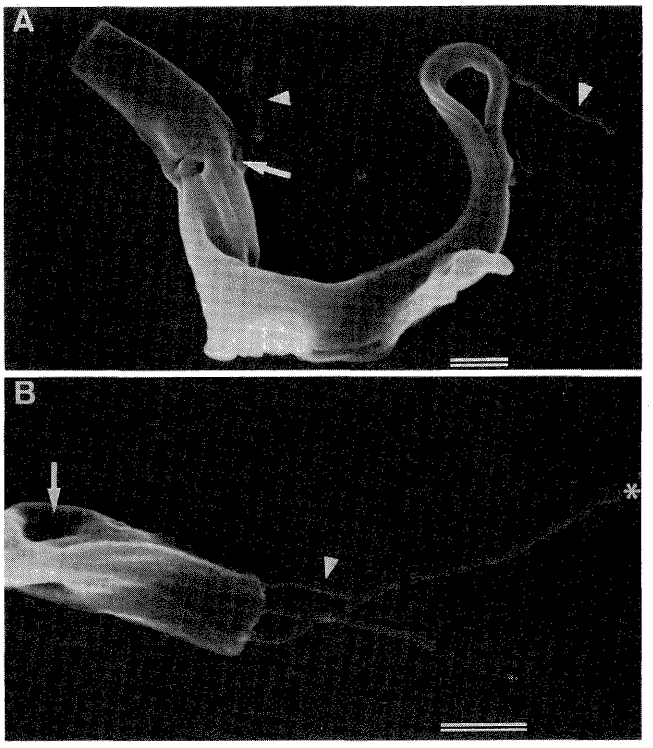

Figure 4 Scanning electron micrographs of akinetoplastic trypanosomes showing filopodia (arrowhead). (A) Filopodia arise from the flagellum at the anterior region and from the posterior portion where it is in close proximity with one of the concavities (arrow) present. The former seems relatively smooth while the latter appears to be segmented. (B) Several filopodia of various length extending from the posterior end of the parasite. The longest appendage appears to be segmented and terminates with a vesicular structure (asterisk). Continuations of the filopodia over the cell surface are shown adjacent to the concavities. Bars, 1 μm.

further confirmed by Southern Blot analysis (Silva-Tahat et al., in press). The mutants were established and cloned in mice, and processed for scanning as well as transmission electron microscopy.

In comparison with *T. brucei,* bloodstream trypomastigotes of *T. evansi* exhibited similar shape, form and pleomorphism as documented by Hoare (1972). While some of them may be long and slender having elongated flagella, some of them are short and stumpy possessing short flagella. These forms have been observed in both kinetoplastic and akinetoplastic clones of *T. evansi.* According to Hoare (1972), the mean measurements of the stumpy, intermediate, and slender forms are 16.8-19.6 μm, 19.5-20.7 μm, and 23.0-24.9 μm, respectively. In the kinetoplastic population,

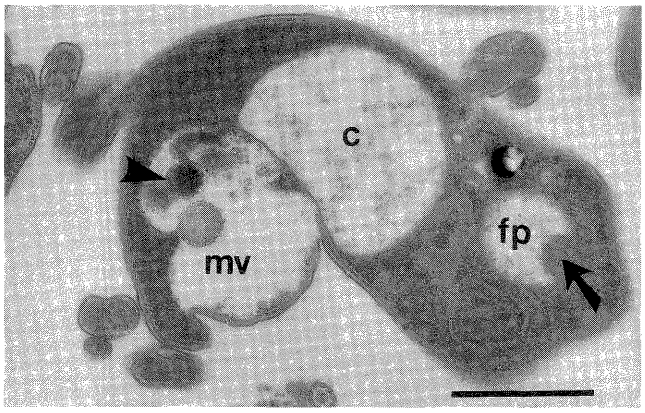

Figure 5 A transmission electron micrograph of an akinetoplastic parasite showing electron-opaque bodies (arrowhead) within the mitochondrial vesicle (mv), the hollow space of the concavity (c), and the flagellar pocket (fp). Note that although the concavity and flagellar pocket appear to be filled with the same amorphous material, the presence of the flagellum (arrow) distinguishes the latter from the former. Bar, 1 μm.

the stumpy form measured 16.8-19.3 μm in length with a mean of 18.3±0.69; the intermediate form was 19.7-21.6 μm with a mean of 20.8±0.65; and the slender form was 22.9-26.1 μm with a mean of 24.1±2.01. On the other hand, within the akinetoplastic population, the stumpy form measured 13.8-19.5 μm with a mean of 17.6±2.94; the intermediate form was 19.5-21.8 μm with a mean of 20.8±0.63; and the slender form was 22.5-24.7 μm with a mean of 23.2±0.99 (representative trypomastigotes are shown in Figure 2). Although there is some degree of variation, these measurements fall within the range of those recorded by Hoare (1972).

The surface of the streamlined akinetoplastic parasite body was essentially smooth and relaxed although a very slight degree of torsion was observed in dividing forms (Figure 3). Of prime interest, however, scanning electron microscopy has illustrated for the first time the presence of concavities or pockets on the surface of more than 90% of akinetoplastic cells in the sample. The orientation and extent of twisting of the remaining akinetoplastic forms did not allow observation of the presence of concavities. The concavities varied in size, depth, number and location on the surface membrane (Figures 2-4). Most were found at the posterior portion of the trypomastigote. An extreme case with respect to size, depth and number of concavity formation was observed in some akinetoplastic cells (AK4). Under the transmission electron microscope, however, they are seen as hollow spaces filled by an amorphous material and lined by the cell membrane (Figure 5). These concavities were apparently absent on the kinetoplastic body surface (Figure 2).

The functional significance of the concavities found on the surface of the akinetoplastic cells is not understood. Nevertheless, pararosaniline was able to induce mutation in the kDNA of the kinetoplastid thereby producing akinetoplastic forms in agreement with the results of Inoki et al. (1962). Other dyes (e.g., acriflavine, ethidium bromide) and antitrypanosomal drugs (e.g., berenil, samorin) have likewise been shown to exert the same effect on other trypanosomes (Hajduk, 1978; Shapiro and Englund, 1990). Notwithstanding, it is also possible that pararosaniline could have produced the concavities. We cannot rule out the possible membrane destabilizing effects of this dye. Drugs such as adenine nucleoside trypanocides (e.g., Puromycin, Cordycepin) and diamidines have been shown to interact with membrane biosynthesis in *T. rhodesiense* (Macadam and Williamson, 1969, 1972). Further studies on the mechanism of action of pararosaniline and scanning electron microscopy of spontaneous mutants may help clarify this observation.

However, despite the presence of concavities, a significant number of akinetoplastic trypanosomes were observed to be in the process of cell division. Fission appears to be initiated at the anterior portion bearing the flagellum and culminated at the posterior broad terminal of the parasite (Figure 3). Longitudinal binary fission is evident. In addition, attached to the posterior (proximal to the concavities) and anterior (usually from the free flagellum) regions of the akinetoplastic bloodstream trypomastigotes are thread-like structures known as filopodia (Figure 4). Filopodia were likewise seen in kinetoplastic parasites (not shown). The filopodia are known to possess the variant surface glycoproteins, or VSGs, (Vickerman and Luckins, 1969) and their formation has been implicated as the shedding of surface antigens (Cherian and Dusanic, 1977). The terminal vesicular structure at the tip of the filopodium (Figure 4B) appears to indicate that it is in the process of rounding off to be subsequently detached from the appendage. Similar vesicles were found in transmission electron micrographs of akinetoplastic forms (not shown).

The concavities were found in (1) established mutants collected from two passages in mice, AK3 and AK4, which have been grown and maintained in the absence of the dye, and (2) both daughter trypomastigotes (Fig. 3B) indicating that they are more likely to be inheritable features of the akinetoplastic clones. Our

observations of akinetoplastic trypomastigotes undergoing cell division and filopodia formation clearly imply that they still perform the fundamental activities of multiplication and infection comparable to the normal parasites. In conclusion, therefore, pararosaniline-induced akinetoplastic clones of *T. evansi* possess harmless unique cell membrane concavities.

ACKNOWLEDGEMENTS

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INTERACTION BETWEEN *BULINUS GLOBOSUS* AND *CLEOPATRA FERRUGINEA* AT A TRANSMISSION SITE OF SCHISTOSOMIASIS IN KWALE, KENYA

SHINICHI NODA[1], NGETHE D. MUHOHO[2], SHIGEHISA HABE[3], KIYOSHI MAKIYA[4], and YOSHIKI AOKI[5]

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Abstract: Snail survey data collected in a small village in Kenya from April 1984 to March 1991 were used to analyze the interaction between *Bulinus globosus* (intermediate host of *Schistosoma haematobium*) and *Cleopatra ferruginea* (unsusceptible snail). An inverse correlation was observed between the two snail populations. This finding leads to the suggestion that *C. ferruginea* has limiting effects on *B. globosus* population. The relative penetrative activity of *S. haematobium* miracidia into the two snail species was also examined. Miracidia penetrated *C. ferruginea* as well as *B. globosus*. Although selective mass-chemotherapy has been repeated every 2 years in our study area, the low infection rates in *B. globosus* were recorded in the year when large numbers of *C. ferruginea* and small numbers of *B. globosus* were collected. Therefore, *C. ferruginea* seems to diminish the transmission of *S. haematobium*; *C. ferruginea* reduce the number of *S. haematobium* miracidia which reach to *B. globosus*.

Keywords: *Bulinus globosus*, *Cleopatra ferruginea*, *Schistosoma haematobium*, snail, biological control, antagonist, decoy

INTRODUCTION

The control of snails that serve as intermediate hosts of schistosomes is an effective means of reducing the transmission of schistosomiasis. However, the use of chemicals to control snails may have undesirable effects on the environment. Therefore, we have become interested in the use of biological control agents that are both environmentally less hazardous and cost effective. Of all biological control strategies, intermolluscan competition is one of the most attractive mechanisms. The ideal biological agent would be able to persist in the habitat of the target snail and induce substantive long-term depression or eradication of the target snail population. Some potential competitor snails which have received considerable attention are *Helisoma duryi*, *Marisa cornuarietus* and *Thiara granifera* (WHO, 1984).

A research program on urinary schstosomiasis has been carried out in a small village in the Kwale District, Kenya since 1981. In our study area, there were some spots where only two snail species, *Bulinus globosus* (intermediate host of *Schistosoma haematobium*) and *Cleopatra ferruginea* were found. The latter species does not serve as an intermediate host for schistosomes or other trematodes of medical or veterinary significance in Kenya (Brown, 1980). We therefore decided to analyze the data obtained so far to examine whether intermolluscan competition occurs at natural snail breeding sites.

As another measure of biological control of schistosomiasis, it has been demonstrated that the infection rate among intermediate host snails exposed to schistosome miracidia was reduced if unsusceptible snail species were present. Such snails apparently prevent miracidia from reaching the intermediate host snails (Chernin, 1968; Upatham and Sturrock, 1973; Laracuente *et al.*, 1979). To determine if *C. ferruginea* has the potential to serve as a miracidial sponge, the present study also examined the relative penetrative activity of *S. haematobium* miracidia into the two snail species.

1 Department of Medical Zoology, Faculty of Medicine, Kagoshima University, Sakuragaoka, Kagoshima 890, Japan
2 Centre for Microbiology Research, Kenya Medical Research Institute, P. O. Box 54840 Nairobi, Kenya
3 Department of Parasitology, School of Medicine, Fukuoka University, Jounan-ku, Fukuoka 814-01, Japan
4 Department of Parasitology and Tropical Public Health, University of Occupational and Environmental Health, Yahata-nishiku, Kitakyushu 807, Japan
5 Department of Parasitology, Institute of Tropical Medicine, Nagasaki University, Sakamoto, Nagasaki 852, Japan

MATERIALS AND METHODS

The study area, Mwachinga village, is located in Kwale District, Coast Province, Kenya. A general description of the study area was given in Noda *et al.* (1988). The Kadingo River flows through the village. It is less than 5 m in width. Some parts of the river dry up in the dry season, leaving small pools. Towards the end of the dry season, these pools sometimes dry up completely. Snail surveys were carried out twice each month at 7 sites along the river. Villagers had frequent contact with water at these sites. The sites were identified as 18B, 18C, 19, 21, 22, 23 and 24. Snails were sampled for 10 minutes by one person using a double-layer steel net scoop (4 mm mesh). The number of each species of snail collected was determined and recorded. *B. globosus* were then put into small petri dishes filled with 2 ml of dechlorinated tap water. The petri dishes were kept in a lighted place for more than 2 hours, and the presence of cercariae of *S. haematobium* was determined under a stereoscopic microscope. Climatic conditions may be classified into four seasons; a long rainy season from April to June, a cool dry season from July to October, a short rainy season in November, and a hot dry season from December to March. Each study period ran from April to March. Snail survey data collected from April 1984 to March 1991 were used to analyze the interaction between the species.

The relative penetrative activity of *S. haematobium* miracidia into *B. globosus* and *C. ferruginea* was examined in the laboratory. *S. haematobium* eggs were collected from school children at Tserezani Primary School in Kwale District. *B. globosus* (13×9 mm) were collected from the Kinango Dam and *C. ferruginea* (16×9 mm) were collected from the Kadingo River in Kwale District. Miracidia were allowed to hatch in dechlorinated tap water. The snails were individually exposed to 20 miracidia in the wells of a 24-well tissue culture plate for 10, 30 and 60 minutes. After exposure, the snails were removed from wells, and the remaining miracidia were counted under a stereoscopic microscope.

RESULTS

Numbers of *B. globosus* and *C. ferruginea* collected monthly in each site are shown in Figure 1. Seasonal changes in total snail population of both species were generally associated with the alternating cycle of rainy and dry seasons. At the end of the long rainy season, snail populations started to increase, reached a peak, and fell during hot dry season. The relative abundance of populations of *B. globosus* and *C. ferruginea* differed by site and year. Therefore, the correlation between annual numbers of *B. globosus* and *C. ferruginea* at each site were analyzed. Inverse correlations were observed between the two snail species. Coefficients of correlation between annual numbers of *B. globosus* and *C.*

Table 1. Annual numbers of *Bulinus globosus* and *Cleopatra ferruginea*, and coefficient of correlation between annual numbers of two species at each site

Period	Species*	Number of snails						
		Site						
		18 B	18 C	19	21	22	23	24
Year 1 (April 1984—March 1985)	*B.g.*	35	393	458	141	390	41	32
	C.f.	678	326	268	399	561	629	2,212
Year 2 (April 1985—March 1986)	*B.g.*	79	606	613	224	831	149	390
	C.f.	165	375	134	104	353	226	1,480
Year 3 (April 1986—March 1987)	*B.g.*	20	255	168	36	422	106	21
	C.f.	735	450	620	465	366	59	812
Year 4 (April 1987—March 1988)	*B.g.*	42	260	260	127	146	54	41
	C.f.	355	166	31	101	409	201	825
Year 5 (April 1988—March 1989)	*B.g.*	442	801	723	389	700	129	282
	C.f.	187	118	48	106	53	141	573
Year 6 (April 1989—March 1990)	*B.g.*	279	458	658	198	706	200	295
	C.f.	89	34	35	87	65	137	301
Year 7 (April 1990—March 1991)	*B.g.*	147	421	475	104	761	159	191
	C.f.	124	13	21	88	68	46	475
Coefficient of correlation		-0.613	-0.237	-0.627	-0.523	-0.650	-0.629	-0.257

* *B.g.*: *Bulinus glogosus*, *C.f.*: *Cleopatra ferruginea*

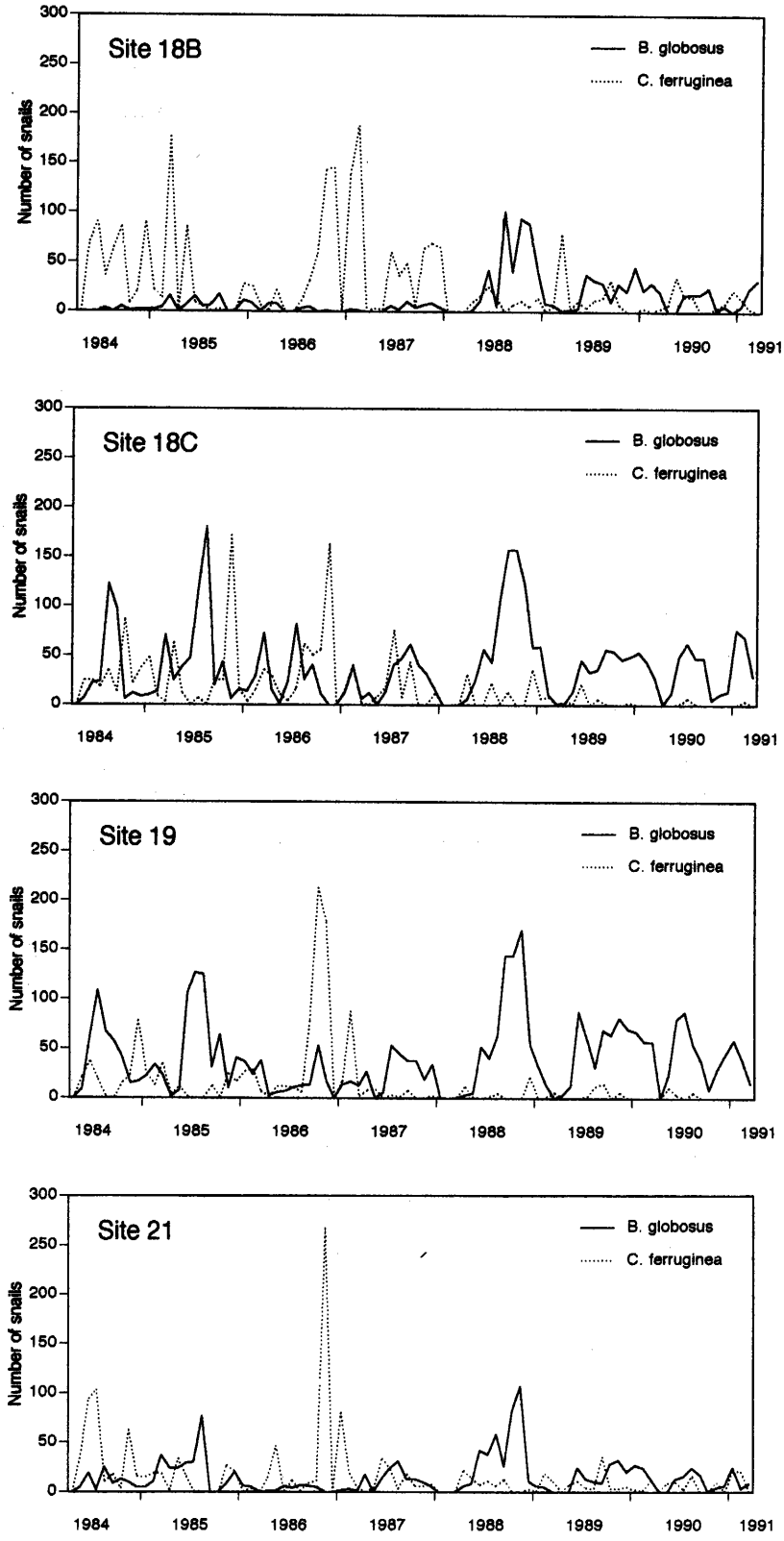
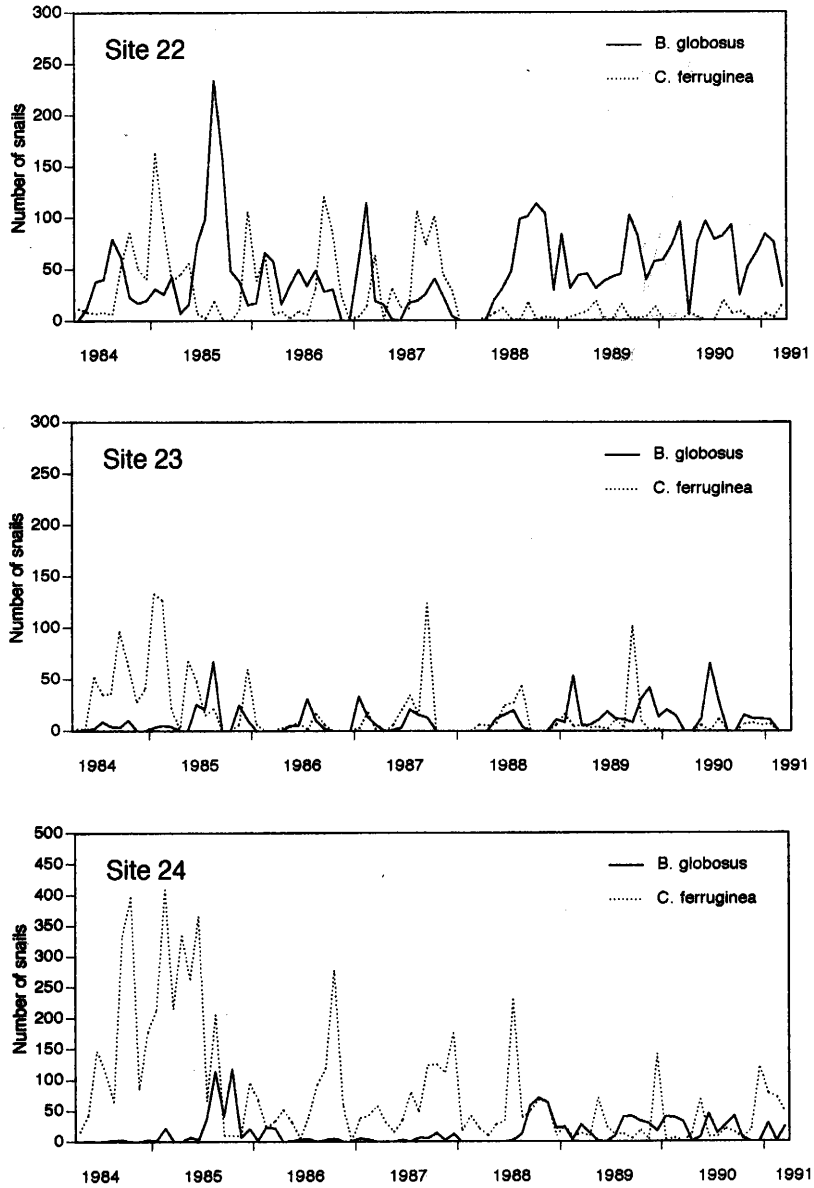


Figure 1. Monthly numbers of *Bulinus globosus* and *Cleopatra ferruginea* collected from 7 sites, April 1984 to March 1991.



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ferruginea varied according to collection sites, but were all negative, ranging from −0.237 to −0.629 (Table 1).

Annual numbers of *C. ferruginea* , *B. globosus* and *S. haematobium* infected *B. globosus* collected at 7 sites are shown in Table 2. In our study area, selective mass-chemotherapy with metrifonate was carried out in February 1984, and that with praziquantel in September 1986, 1988 and 1990 (Sato *et al.*, 1988; unpublished date). Whenever mass-treatment was given, the overall prevalence in our study area fell rapidly after treatment, but rose again after 1 year to the pre-treatment level. Therefore the level of contamination of river in year 3 was comparable to that of years 5 and 7, and the level in year 4 was comparable to that of year 6. The infection rate of year 3 was lower than those of year 5 and 7, and that of year 4 was lower than that of year 6. Years 3 and 4, when the lower infection rates were recorded, were those when large numbers of *C. ferruginea* and small numbers of *B. globosus* were collected.

The results of the experiments on the relative penetration of miracidia into *B. globosus* and *C. ferruginea* are shown in Table 3. Duming the early period of snail-miracidia contact, *B. globosus* was penetrated by a higher number of miracidia than *C. ferruginea* ($P < 0.05$, Student's t-test). Later, however, *C. ferruginea* was also penetrated by a high number of miracidia.

Table 2. Annual numbers of *Cleopatra ferruginea, Bulinus globosus*, and *Schistosoma haematobium* infected *B. globosus* collected from 7 sites

Period	Number of snails		
	Cleopatra ferruginea	*Bulinus globosus*	Infected *B. globosus* (infection rate)
Year 1 (April 1984—March 1985)	5,073	1,490	71 (4.8%)
Year 2 (April 1985—March 1986)	2,837	2,892	114 (3.9%)
Year 3 (April 1986—March 1987)*	3,507	1,028	2 (0.2%)
Year 4 (April 1987—March 1988)	2,088	930	1 (0.1%)
Year 5 (April 1988—March 1989)*	1,226	3,466	111 (3.2%)
Year 6 (April 1989—March 1990)	748	2,794	61 (2.2%)
Year 7 (April 1990—March 1991)*	835	2,258	25 (1.1%)

* The selective mass-chemotherapy with praziquantel was carried out in September 1986, 1988 and 1990.

Table 3. Number (Mean ± S.D., N=6) of *Schistosoma haematobium* miracidia remaining after exposure to *Bulinus globosus* and *Cleopatra ferruginea* (Snails were individually exposed to 20 miracidia)

Species	Minutes of exposure		
	10*	30	60
Bulinus globosus	10.1±3.3	7.7±2.4	7.8±2.9
Cleopatra ferruginea	14.7±2.7	11.8±4.7	9.8±3.8

* Number of remaining miracidia exposed to two snail species are significantly different at the 0.05 level (Student's *t*-test).

DISCUSSION

T. granifera and *T. tuberculata*, belong to the gastropod family Thiaridae, may be effective competitors with *Biomphalaria* (reviewed by Pointier and McCullough, 1989). The potential of *T. granifera* in biological control has been investigated in St. Lucia. In four field trials, *B. glabrata* was apparently eliminated from marshes and streams 6 to 22 months after the introduction of *T. granifera* (Prentice, 1983). In Martinique, *T. tuberculata* was introduced into two groups of watercress beds, and both *B. globosus* and *B. straminae* were eliminated from the transmission sites within 3 years of the introduction of the competitor (Pointer *et al.*, 1989). Mkoji *et al.*(1992) reported that *Melanoides tuberculata* (Thiaridae) co-exists with *Biomphalaria pfeifferi* and other pulmonates in Kenyan freshwater habitats, and possibly acts to regulate pulmonate populations. After molluscicide application, all four snail species (*M. tuberculata, B. pfeifferi, Lymnaea natalensis* and *B. globosus*) became scarce. Then, *B. pfeifferi* populations recovered and achieved levels of relative abundance considerably higher than noted prior to molluscicide application; *M. tuberculata* was slow to recover following molluscicide. In our study area, *C. ferruginea*, also a member of the Thiaridae, was present and was locally abundant. An inverse correlation between populations of *B. globosus* and *C. ferruginea* was observed in a natural setting. The data suggest that *C. ferruginea* has limiting effects on *B. globosus* populations. It is likely that *C. ferruginea* destroys eggs and young snails of the vector and competes with vector snails for space and foods.

In addition to the possible role of *C. ferruginea* as an effective antagonist to vector snails, the presence of unsusceptible snails to schistosome may reduce the infection rate of snails by acting as a miracidial sponge or as a decoy (Chernin, 1968; Upatham and Sturrock, 1973; Laracuente *et al.*, 1979). Upatham and Sturrock (1973) suggest that unsusceptible snails have a significant effect on diminishing transmission, especially when chemotherapy has been used to reduce the worm burden and egg load in the human population. The experiments on the relative penetration of miracidia into *B. globosus* and *C. ferruginea* showed that *S. haematobium* miracidia also penetrated *C. ferruginea*. In our study area, the low infection rates in *B. globosus* were recorded in years 3 and 4 when large numbers of *C. ferruginea* and small numbers of *B. globosus* were collected; the infection rate in year 3 was lower than those in year 5 and 7, and the infection rate in year 4 was lower than that in year 6. As was described previously, the level of contamination of water at year 3 and 4 was comparable to that of year 5 and 7, and that of year 6 respectively. Our study may indicate that the predominance of *C. ferruginea* over *B. globosus* reduces the infection rate of snails by reducing the number of *B. globosus*, and, in addition, by reducing

the number of miracidia which reach to *B. globosus*.

C. ferruginea is widely distributed in permanent rivers and muddy residual pools in seasonal streams along the east coast of Africa (Kenya, Tanzania, Mozambique and South Africa) where *S. haematobium* is endemic (Brown, 1980). *C. ferruginea* may have a previously unappreciated effect on diminishing the transmission of *S. haematobium* in these coastal habitats. *C. ferruginea* appears to present interesting possibilities as an effective tool for biological control. Further investigations should be attempted to examine this possibility.

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A STUDY OF FATIGUE COMPLAINTS AMONG WORKERS EXPOSED TO HEAT STRESS IN AN ELECTRONIC COMPANY IN HANOI, VIETNAM

SHINYA MATSUDA

Received November 28, 1994/Accepted January 30, 1995

Abstract: The relation between exposure to heat stress in the workplace and the degree of fatigue complaints was investigated by a questionnaire (composed of 30 items) among 39 assembly line workers in an electronic factory in Hanoi, Vietnam. There is a considerable difference in the change of fatigue complaints before and after work between workers exposed to heat and those not exposed. Among the workers not exposed to heat stress, the rate of fatigue complaints increased in 8 items, all of which did not show any statistically significant change during the work. In the case of workers under heat stress, the rate increased in 25 items, of which nine showed statistically significant increase after working hours. Especially, the changes were more apparent among the items corresponding to 'drowsiness and dullness'. A special consideration must be paid to heat stress in tropical countries such as Vietnam, in order to prevent occupational impairment.

INTRODUCTION

After the introduction of the new open market policy in 1986, Vietnam has begun to realize a rapid economic growth. Especially, the permission for foreign companies to invest in the country has made it possible to organize a number of joint ventures between Vietnam and other countries. It is expected that the living conditions of the Vietnamese will be improved gradually by the stabilization of the national economy. However, this rapid economic development may cause an intensification of labor, especially in the newly developed factories, most of which are labor intensive sectors, such as textile industries and food processing factories. Various "newly adopted" work conditions (i.e., shift work, assembly line, etc) have been adopted in these workplaces, where the standards of labor safety and hygiene are not always respected[1]. As a result, various health problems at the workplaces have emerged in addition to the traditional occupational health problems (i.e., silicosis, saturism, etc). To evercome these problems, much attention to occupational safety from the ergonomic point of view must be paid in the developing countries like Vietnam. It is well known that most occupational accidents occur due to the lack of attention of workers and that a mentally exhausting situation is very apt to cause severe occupational accidents. In tropical countries like Vietnam, the exhausting working conditions are very energy consuming both physically and mentally. Therefore, a special consideration should be given to the working conditions in a tropical environment. In this report, I present the results of studies focusing on the relation between the heat stress and complaints of fatigue of assembly line workers in an electronic company in Hanoi, Vietnam.

STUDY AND METHODS

An electronic company in Hanoi, Vietnam was selected for the study. This factory, which was established as a joint venture between Korea and Vietnam, produces 10 thousand television sets per month on an assembly line. The average working time is from 8:00 am to 5:00 pm (there is a two-hour rest at lunch time). The two most important origins of heat in the factory are the workplaces where the assembled monitor is checked, and where the soldering machine is located.

Thirty-nine assembly line workers were investigated regarding their working conditions as well as fatigue complaints before and after work, by using two questionnaires. All questionnaires were completed by the workers themselves. The first questionnaire was prepared in order to obtain background information such as; age, sex, educational level, living conditions (i.e., size

Department of Preventive Medicine and Community Health, University of Occupational and Environmental Health, Japan

of house, number of family members), monthly income, distance between residence and factory, hazardous conditions at the workplace (i.e., heat, noise, chemicals, radiation, etc). The second questionnaire was the fatigue questionnaire developed by the Japan Association of Industrial Health, which is composed of 30 questions under three categories; the first 10 items are related to "Drowsiness and dullness", the second 10 items to "Difficulty in concentration", and the last 10 items to "Projection of physical disintegration"[2]. The questionnaire was administrated before and after work in order to monitor the changes in subjective fatigue symptoms during the working hours.

The basic statistics of the workers are as follows:
Sex distribution: 28 males and 11 females,
The average age of workers: 26.8 (SD=8.0, Min=15, Max=48),
Number of workers exposed to heat: 28

At first I conducted an analysis stratified by sex, but as there was no difference between the two, I will show the result of combined sex analysis in order to

Table 1 Changes in the rate of those who had corresponding fatigue complaints before and after work in an electric company at Hanoi, Vietnam (stratified by the existence of heat stress, n=39)

Complaints	Heat(−) (n=11)			Heat(+) (n=28)			Significance of difference due to heat**	
	Before work (%)	After work (%)	Significance of change*	Before work (%)	After work (%)	Significance of change*	Before work	After work
I. Drowsiness and dullness								
1 feel heavy in the head	9.1	18.2	p=0.317	25.0	67.9	p=0.005	p=0.262	p=0.005
2 get tired of the whole body	36.4	63.6	p=0.109	32.1	82.1	p=0.004	p=0.801	p=0.205
3 legs feel heavy	0.0	9.1	p=0.317	0.0	17.9	p=0.043	N.C.	p=0.447
4 give a yawn	18.2	18.2	p=1.000	21.4	28.6	p=0.361	p=0.821	p=0.409
5 feel the brain muddled	18.2	18.2	p=1.000	0.0	21.4	p=0.028	p=0.074	p=0.599
6 become drowsy	18.2	45.5	p=0.109	35.4	53.6	p=0.139	p=0.286	p=0.648
7 feel strained in the eyes	36.4	36.4	p=1.000	32.1	64.3	p=0.008	p=0.801	p=0.111
8 become rigid or clumsy in motion	0.0	9.1	p=0.317	0.0	14.3	p=0.068	N.C.	p=0.562
9 feel unsteady in standing	0.0	0.0	p=1.000	0.0	0.0	p=1.000	N.C.	N.C.
10 want to lie down	9.1	0.0	p=0.317	3.6	14.3	p=0.109	p=0.490	p=0.249
Average number of items marked in I	1.5	2.2	p=0.054***	1.5	3.6	p=0.000***	p=0.949***	p=0.066***
II. Difficulty in concentration								
11 feel difficult in thinking	0.0	0.0	p=1.000	7.1	17.9	p=0.109	p=0.510	p=0.171
12 become weary of talking	27.3	27.3	p=1.000	10.7	28.6	p=0.091	p=0.197	p=0.632
13 feel irritable	9.1	9.1	p=1.000	10.7	17.9	p=0.180	p=0.687	p=0.447
14 unable to concentrate attention	0.0	0.0	p=1.000	0.0	0.0	p=1.000	N.C.	N.C.
15 become apt to forget things	18.2	9.1	p=0.317	7.1	28.6	p=0.028	p=0.312	p=0.194
16 unable to have interested in things	9.1	9.1	p=1.000	3.6	7.1	p=0.317	p=0.482	p=0.642
17 feel uneasy about things	0.0	0.0	p=1.000	3.6	7.1	p=0.317	p=0.718	p=0.510
18 apt to make mistakes	9.1	0.0	p=0.317	25.0	28.6	p=0.317	p=0.262	p=0.051
19 unable to straighten up in the posture	27.3	27.3	p=1.000	10.7	28.6	p=0.043	p=0.208	p=0.632
20 no energy	0.0	9.1	p=0.317	0.0	0.0	p=1.000	N.C.	p=0.282
Average number of items marked in II	1.0	0.9	p=0.676***	0.8	1.6	p=0.001***	p=0.696***	p=0.202***
III. Projection of physical disintegration								
21 have a headache	27.3	36.4	p=0.317	25.0	53.6	p=0.025	p=0.884	p=0.333
22 feel stiff in the shoulders	9.1	9.1	p=1.000	21.4	39.3	p=0.091	p=0.346	p=0.068
23 suffer low back pain	0.0	9.1	p=0.317	25.0	46.4	p=0.059	p=0.077	p=0.030
24 feel oppressed in breathing	0.0	0.0	p=1.000	3.6	17.9	p=0.068	p=0.718	p=0.171
25 feel thirsty	27.3	27.3	p=1.000	17.9	39.3	p=0.059	p=0.400	p=0.356
26 have a husky voice	0.0	0.0	p=1.000	3.6	3.6	p=1.000	p=0.718	p=0.718
27 have a dizziness	9.1	0.0	p=0.317	7.1	32.1	p=0.018	p=0.642	p=0.033
28 have a spasm on the eyelids	0.0	0.0	p=1.000	0.0	3.6	p=0.317	N.C.	p=0.718
29 have a tremor in the limbs	0.0	0.0	p=1.000	3.6	10.7	p=0.180	p=0.718	p=0.358
30 feel unwell	0.0	0.0	p=1.000	0.0	0.0	p=1.000	N.C.	N.C.
Average number of items marked in III	0.7	0.8	p=0.341***	1.1	2.5	p=0.001***	p=0.515***	p=0.019***
Average number of items marked in Total	3.2	3.9	p=0.195***	3.4	7.8	p=0.000	p=0.915***	p=0.032***

*: Wilcoxon test (Before and after), **: Chi square test or Fisher's exact test (Heat + vs. Heat −), N.C.: Not calculated, ***: t-test

increase the statistical stability of the analysis. The changes in fatigue complaints were analyzed before and after work stratified by the existence of heat stress (Wilcoxon test). The differences in the positive rate of fatigue complaints were compared between workers under heat stress and those not exposed (Chi square test, Fisher's exact test and t-test). The SPSSX statistical package in the computer system of UOEH was employed in this investigation.

RESULTS

Table 1 presents the results concerning changes in subjective fatigue complaints before and after work, stratified by the existence of heat stress in the workplace. There is a considerable difference in the changes of fatigue complaints between two conditions. Among the workers with no heat stress, the rate of workers who had corresponding fatigue complaints increased in only 8 items, all of which did not show any statistically significant change between before and after work. In the case of workers exposed to heat, the rate increased in 25 items, of which nine showed a statistically significant increase after work. Especially, the changes were more apparent among the items of 'Drowsiness and dullness' (i.e., feel in the head, get tired of the whole body, legs feel heavy, feel the brain muddled, feel strained in the eyes). When the average number of marked items before and after work in each three major categories as well as in total was compared, a statistically significant increase was observed among the workers under heat stress in all categories (I: $p=0.000$, II: $p=0.001$, III: $p=0.001$, Total: $p=0.000$; t-test), but not among the workers without the stress. When the number of marked items in each category and in total were compared between the two heat exposure conditions before and after work, respectively, the numbers of the two conditions before work were almost the same, but the numbers of marked items were larger among the workers exposed to heat with statistical significance after work (I: $p=0.066$, II: $p=0.202$, III: $p=0.019$, Total: $p=0.032$; t=test).

DISCUSSION

According to the present results, the existence of heat stress at the workplace is significantly associated with the increase of fatigue complaints among the assembly line workers investigated in my study. In Vietnam, special attention should be paid to the existence of heat stress in factories in order to prevent occupational accidents caused by exhaustive conditions of a worker due to heat stress, because heat imbalance may occur even though there is only a slight increase in temperature in a tropical country like Vietnam. The relationship between heat loss/gain variables and internal heat production is described by a simple heat balance equation[3]:

$$S=(M-W)\pm C\pm R-E,$$

where S=net amount of heat gained or lost by the body,
M=heat production by metabolism,
W=external work performed,
C=heat transfer by convection,
R=heat transfer by radiation, and
E=body heat loss by evaporation.

In a tropical country, because the environmental temperature is frequently higher than the surface body temperature, radiation and convection can increase the body temperature. Furthermore, in an extremely warm-moist environment, as found in textile factories located in tropical countries, a more troublesome situation usually arises, where the heat loss by evaporation is completely disturbed. For example, a heat collapse and a heat stroke may easily occur in such a situation. Luong reported that workers in textile factories in Vietnam are usually exposed to temperatures of about 39-40 °C with 80% humidity in average[4]. In the present case, workers under heat stress are working in an environment 1 or 2 °C higher (33-34 °C) than the outdoor temperature, with 80% humidity (same as the outdoor humidity). Therefore, although we could not detect any severe cases of heat-related illnesses, mild forms of impairment (i.e., irritability, lassitude, decrease in morale, and inability to concentrate) due to the prolonged exposure to the moderately hot climate were observed. This situation can be dangerous in workplaces, especially in a place where enough attention to the occupational safety is not always paid, as is often observed in developing countries like Vietnam. Systematic studies on the effects of exposure to uncomfortably warm environments have clarified that the number of accidents, sickness and labor turnover increases because of their chronic effects on the central nervous system. Inefficiency due to the increase of minor accidents, errors, and slower work rate is also another important problem for developing countries who intend to realize a rapid economic development. In the current situation, both the Vietnamese workers and their employers do not seem to pay any special attention to occupational safety and hygiene such as heat, noise, and other harmful substances (i.e., heavy metals, organic solvents and

toxic gas), although there is a special regulation legislated by the government for this purpose[5]. It is natural that a rapid economic development is being given priority in order to stabilize the society in the current situation of Vietnam. In such a situation, it is very difficult to give adequate consideration to the working conditions. According to the results of another questionnaire[6], most of the workers, even though they are exposed to some occupational hazards have answered that they are satisfied with the present working conditions, because a stable monthly income is assured, even if there is a possibility of some occupational health impairments in the future. Forty percent of the workers have replied that they will endure some occupational risks to their health in order to earn money to live[6]. As experienced in Japan, the ignorance of occupational safety and hygiene will have drastic results in the long run. Therefore, special attention must be paid to occupational safety and hygiene from the early stage of industrial development.

A number of studies have been conducted in developed countries, in order to determine the TLV (Threshold Limit Values) of various occupational risks, including temperature at the workplace. For example, the desirable temperatures in the workplace are set as follows; 30.6 °C (Wet Bulb Globe Thermometer: WBGT) for light work (235-405 J/s), 28.0 °C (WBGT) for moderate work (up to 235 J/s)[7]. However, it is not appropriate to apply these standards to developing countries in the tropics, because they have been developed in western countries, most of which are in the temperate zone. There is a difference in heat tolerance and comfortable temperature zone for inhabitants of the tropics and those of other regions. Therefore, different standards must be established in considering the physical and social characteristics of the Vietnamese. However, it is not easy for the Vietnamese researchers to accomplish this task because of the shortage of money, materials and manpower. It is expected that international collaborative programs in the field of occupational safety and hygiene will be organized in the near future. Finally, in addition to the epidemiological research like the present one, physiological studies must be conducted to establish standards for improving the working condition in Vietnam.

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This study was conducted with a financial assistance from the Nissan Science Foundation. The author wishes to express his deep gratitude to Mr. N.A. Luong (Director of the National Institute of Labour Protection, Vietnam) for his warm-hearted assistance for the present study in Vietnam. The author is also grateful to Prof. Kahyo (Department of Preventive Medicine and Community Health, U.O.E.H.) for his helpful criticism.

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PROCEEDINGS OF XXXVI ANNUAL MEETING OF JAPANESE SOCIETY OF TROPICAL MEDICINE

1-2 December 1994, Kagoshima

President

Yoshihito Otsuji

(Director General : Kagoshima Prefectural Comprehensive Health Center)

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Prize winner's lecture

JSTM (Japanese Society of Tropical Medicine)
Young Investigator Award

ANTIBODY FREQUENCY DISTRIBUTION CURVE FOR RISK ASSESSMENT OF A MALARIA EPIDEMIC

SHIGEYUKI KANO
Department of Parasitology, Gunma University School of Medicine

Seroepidemiology is important, in that it provides more informative *period prevalence* data which cannot be obtained through microscopy. In particular seroepidemiological assessment is valuable when a malaria control program reaches the more advanced stage and the endemicity of malaria bocomes very low in the controlled areas. On such an occasion, a more suitable method for epidemiological surveys is required, in order to detect latent malarial foci in apparently controlled areas. In the present study, follow up epidemiological surveys were conducted in two villages in the Sudan in 1987 and 1989. In 1987, the frequency distribution curve of antibody titers, which was measured by the ABC-ELISA, revealed a potential danger of future malaria epidemics in one of the villages, Sennar, showing a bimodal pattern with a second peak at titer 1:512. The subjects who manifested titer of 1:512 were those considered to have contracted malaria about the time when the survey was done, and they might constitute the probable focus of succeeding transmission in the community. Thus the second mode of the curve was regarded as the significant indicator of potential risk of malaria epidemics which might occur as a consequence of environmental or man-made changes. This assumption was proven to be true after the disastrous flood occurred in August 1988, which adversely affected the well implemented malaria control program in the whole area including our study villages. In the study in 1989, increased malaria prevalence was actually observed by microscopy. The antibody titers obtained in Sennar changed into hyperendemic pattern, showing the highest peak at the highest titer 1:1024. This pattern was the reflection of the very recent past or current infection in the majority of the people at the time of the survey. On the other hand in Mobi, the other village under study, the frequency distribution curve of the titer, characterized by a single low-titered peak in 1987, changed into a bimodal pattern suggestive of a small, recent past outbreak of malaria in the village in 1989. Comparison of seropositivity and slidepositivity rates did not present significant difference between the results before and after the flood. It was thus affirmed in this study that the actual prevalence of malaria may be more precisely reflected in the shape of the frequency distribution curve of antibody titers. Application of this method will be of considerable value in identification of early or even presumptive malaria foci where control measures must be concentrated.

We also applied this method of malaria assessment to an indian colony located in the Amazon jungle, where malaria was regarded as a minor disease by the inhabitants. The reported incidence was very low, and in fact, no parasitemia was detected in the blood smears of a group of inhabitants obtained through our short visit. But on the contrary, the ABC-ELISA revealed a 100% prevalence of malaria antibodies in the population, and the frequency distribution curve of the titers showed a bimodal pattern suggesting that malaria transmission was actively taking place in the colony. Therefore, serological studies which can detect the *period prevalence* are useful for the assessment of malaria situation in the highly endemic community which is not readily accessible, or whose populations seem to have acquired a certain degree of immunity depressing parasitemia to submicroscopic levels.

The wider application of seroepidemiology is envisioned in defining foci in an endemic area in Palawan, the Philippines, where appropriate control campaigns are to be conducted. In the community under study, there was a significant difference in the geometric mean reciprocal titer during the rainy season as compared with that during the dry season. Seasonal changes in the distribution of high- and low-titer responses, which is suggestive of the occurrence of recent past malaria epidemics, were also recognized. Using this method of malaria assessment, we could also identify the focus even in a limited area, supporting the observation that malaria is not evenly spread over the geographic areas in which it is prevalent, instead, it is highly focal.

Thus, the three studies described above definitely show the significance of the application of antibody frequency distribution curve in malaria seroepidemiology, particularly in generating more precise and informative malariometric data which is critical in successful malaria control.

Special lecture

How to attain the Control of Tuberculosis in the Tropics

NOBUKATSU ISHIKAWA
(The Research Institute of Tuberculosis)

Global TB Problem

Tuberculosis (TB) is still one of the major killing diseases in the world 8 million new cases and 3 million deaths every year. It can be a priority tropical disease as more than 70% of those new cases or dead cases occur in the tropics. Few more points need to be mentioned for the problem of TB; 1) It has been neglected in the past few decades in most of the developing countries; 2) Most productive and working age groups are affected; 3) The epidemiological situation is being deteriorated in most of the areas partly due to HIV infection; 4) New move to strengthen the global TB control program is urgently needed as the situation would continuously deteriorate if no measures are taken; 5) Tuberculosis control is one of the most cost-effective measures according to the recent analysis by the World Bank.

Why does TB not decrease in developing countries?

The possible reasons for no reduction or increase of TB problem in most of the countries in the tropics are; ①biomedical characteristics of TB as a chronic disease, ②insufficient socio-economic development, ③poor health infrastructure, ④poor TB program, and ⑤new epidemic of HIV infection.

Considering the current epidemic situation of HIV infection, strengthening of TB control is urgently needed in most of the countries in the tropics under the intensified international cooperation scheme.

New Framework for TB Control Program

WHO has recently issued a policy package for effective TB program based on the analysis of success cases in TB control. The target should be first to establish 85% cure rate of the sputum smear positive patients, and then to develop 70% case-finding coverage. To attain these, a following package program is needed; i. e. ①government commitment, ②case finding by sputum microscopy with its network and quality control, ③adoption of short course chemotherapy, ④ secured supply of anti-TB drugs, ⑤standardized monitoring system for treatment.

However practice is not easy. In macro scale, international financial and technical assistance is needed and in micro scale, primary health care system and community participation need to be promoted. Moreover trials in pilot areas need to be made to develop a suitable system to each country.

From the author's experience

Sixteen years experiences in Bangladesh by the author are demonstrated including participatory action research for the integration of TB program into Thana (sub-district) general health services. In the international cooperation, the ultimate goal is not only the technology transfer but further system development with full participation of the staff of the country, resulting in the empowerment of the people concerned in problem finding and its solution.

Special lecture

OVERSEAS EXCHANGE BY SATSUMA HAN

IZUMI HARAGUCHI
Faculty of law and literature, Kagoshima University

島津77万石と俗に言われるが，江戸時代の薩摩藩は，宮崎県の南半分から琉球王国の与那国島までを含む，ちょうど日本の本州の長さに匹敵する領域だった。このうち琉球王国は，薩摩藩が支配しながらも，国際的には立派な独立王国だった。そこに，薩摩藩の海外とのつながりが出てくるわけである。

ＮＨＫの大河ドラマ「翔ぶが如く」の時代考証をした時に，明治維新の薩摩藩の原動力は何だったのか，その放送だけでは本当の理解は得られないという思いがあって，ＮＨＫ側に「琉球の風」を茶の間に届けてほしいと願い出た結果取り上げられた。私は琉球の風が吹いて，翔ぶが如くに明治維新が成就したという考えで，この二つのドラマの時代考証をした。

琉球ルートを通じて，中国の学問，芸術，文化が薩摩に入って来たことは間違いない。長崎は，海外からの商品の窓口だったのに対して，琉球王国と中国とは，国家と国家の交流である。中国福州に琉球館があり，常時100人以上の琉球の人たちがいた。そこに高嶺徳明（中国名・魏士哲）という館付きの通訳官がいた。徳明は，中国人黄会友から手術の秘法を伝授された。徳明が琉球に戻った頃，王国の尚貞王の11歳の孫尚益が，生まれながらの兎唇だった。徳明はその手術をしたのである。1689年のことである。

福州で得た技術で，琉球王の孫に手術を施したということで，薩摩藩が聞き逃がすはずはない。琉球在番奉行付きの医者・伊佐敷道与（1661—1730）が，高嶺徳明から学んで薩摩に帰ったあと，川内川中流域の宮之城佐志領主であった島津家分家の23歳の子息に手術を施したという記録がある。川内市の歴史民俗資料館に，道与の弟子が書いた秘伝書が残っているが，この手術は「酸鼻余りに忍びず」と書かれていて，ものすごい手術だったことがわかる。華岡青洲が，全身麻酔術を施して乳がんの手術をしたのが，その70年後であることを考えると，薩摩の海外とのつながりを示すよい事例だと思うが，全身麻酔がどれほど効いていたのか，麻酔を全くしないで行ったのではなかろうしと，私も確信は持てない。

さかのぼって，関ケ原の戦いで奮戦したのが，加治木に隠居していた島津義弘である。城下町の加治木になぜ医者が多いのか。これは，加治木が海外との貿易港であったことと，戦国時代いつも金瘡手術をしなければならなかったからではなかろうか。島津義弘自身，金瘡手術を心得ていたという記録がある。生涯50回の戦をした人だから，金瘡手術を心得ていなければ，命永らえなかったと思われる。

義弘の時に，島津氏は徳川に負けた。しかし，薩摩藩はその幕府を倒し，最後の将軍徳川慶喜は，大政を奉還した。その時点で，五代友厚，寺島宗則らの藩費留学生が海外に赴き，わが国に初めて近代的な学術をもたらした。その流れの中で，明治２年，ウィリス博士が呼ばれ，鹿児島の外科術がイギリス流として芽生え，鹿児島大学医学部へと発展した。ウィリス博士は，西南戦争直前までいて，治療した人は一万人以上にのぼっている。また門弟の一人が慈恵医大を創設した高木兼寛先生で，先生はやはりイギリス流の医学者である。臨床を常に重視し，地域の課題に応じるというその伝統は，学術的な炎を灯しつづけながら受け継がれ，この日本熱帯医学会も，東南アジア，東アジアにおける本当の意味の国際貢献を果たしていると思う。

Special lecture

ESTABLISHMENT OF SUPPLY SYSTEM OF ORPHAN DRUG FOR TROPICAL DISEASES AND ITS SUPPORTING MEASURE BY THE MINISTRY OF HEALTH AND WELFARE

HIROSHI OHTOMO
Department of Tropical Medicine, Jikei University School of Medicine

There is no doubt that drugs have made a considerable contribution to the fight against a whole series diseases. However, one realized that there are some parasitic infections or tropical diseases of which drug treatment leaves a lot to be desired or is even to totally inadequate, because of no available in Japan. In the last 20 years, patients with so-called imported tropical diseases were increasing gradually in Japan as results of the changes of life style and the internationalized personal interchanges. At 1980, the Group for the study of the drug treatment of imported tropical diseases based on the research enterprise by the Ministry of Health and Wealfare was founded and established an access route of orphan drugs for parasitic infections and tropical diseases for physicians by free distribution at their requests: 15 kinds of orpahn drugs were imported and confirmed qualifications for the drug under investigations by National Institute of Hygienic Sciences. At 1988, this study group was reorganized into the Group for the study of the drug development for tropical diseases. Then, over 3,000 patients with parasitic infections such as malaria, strongyloidiasis, amebiasis, echinoccosis and *Pneumocystis carinii* pneumonia were treated successfully by orphan drugs supplied from these study group. In addition, supporting measures in Japan for the development of orphan drugs were operated on the basis of a pharmapeutical affairs bureau's notification at 1985,on affix materials concerning an applicatin for permit of production(import) of orphan drug. Under this mearure, six orphan dugs, thiabendazole, sulfadoxine/pyrimethamine, praziquantel, mebendazole, pentamidine, albendazolo, had been approved for clinical use by the Ministry of Health and Welfare.

At April, 1993, a proposed amendment of the Drugs, Cosmetics and Medical Instruments Act and the Promotions Foundation Act for Relief and Study of Adverse Drug Reactions was passed at the 126th session of the Dict. Then, with starting research and development promotion system from October, above two study groups were re-reorganized into the Group for the study of the drug development enterprise. At present, this study group is developing works on supply of orphan drugs for tropical diseases and clinical investigatitions of these drugs. This paper briefly covers the achievement of orphan drugs supply, and internal and external circumstance of these drugs.

Special Lecture

A JUST SIMPLE ZEAL-MY PAST 30 YEARS IN PURSUIT OF FILARIAL CONTROL

YOSHIHITO OTSUJI, M.D.
Kagoshima Prefectural Comprehensive Health Center

Filariasis had formerly been prevalent over all Japan, mainly in Southern Kyushu. In one of our research activities, we tackled control of filariasis. Results of our filarial control work are described hereinafter.

1) History of World Filariasis:
In around B.C. 1500 the disease was already known in Egypt in the form of frescoed elephantiasis. In Japan, on the other hand, elephantiasis of the lower extremities and scrotal hydrocele were found drawn in a picture scroll in around 1000. Further, the state of filarial spread in Japan was reported based on varied historical materials.

2) Signs and Symptoms of Filariasis:
We have seen elephantiasis cases of huge penis, scrotum, the upper and lower extremities and the labium and scrotal hydrocele and chyluria, etc.

3) Treatment of Filariasis:

Our treatment study was focused on diethylcarbamazine (DEC or Supatonine) regimen (dosage, effectiveness and side effects, etc.), which later was confirmed to have long-lasting effectiveness for remarkable reduction of microfilaria (mf) count.

About 1,500 people living in district of Kiyohara, Kagoshima prefecture, were also subjected to our mass treatment project, which lowered the mf positives almost completely to zero level among them.

4) Mass Treatment of Filariasis Patients:

Japanese Central Government-sponsored filariasis prevention project was started in 1962. Our concluded standards for most effective method of filarial control are as follows:

a) Blood to be taken after 9:00 P.M.
b) Blood quantity to be taken is 30cmm.
c) About 100 people are reasonable number per one medical staff for blood taking.
d) Total dose of DEC is 70mg/kg/B.W. per person.
e) Continued and intermittent administration of DEC to be combined.
f) Side effects of DEC administration do not require any preventive measures. The subjected people, however, should be kept fully-informed on the side effects, especially on possible fever onset.
g) Subjected people should be well-explained about filariasis in order to obtain their full cooperation. Exhaustive public relations are to be promoted in this connection.

About 15 or more years were required to reach above-mentioned conclusions. We further have come to know that on-the-spot work in order to confirm the righteousness of our knowledge was needed to overcome miscellaneous problems.

5) Mass Treatment Intended Mainly to Eradicate Infection Sources by Administration of DEC:

The filarial prevention project covered one metropolis and 8 prefectures, where 34,350 mf positives were detected among 2,039,728 people examined. In Kagoshima 27,450 mf positives were found out of 771,924 examined during 1962-1971 period. About 80% (27,456) of all mf positives (34,350) were found in Kagoshima.

6) Follow-up Results of Mass Treatment with DEC:

Follow-up investigations were made after 10 years on people treated with DEC in Kiyohara district, Bounotsu-cho, Kagoshgima and Kuro island of Okinawa prefectures, which revealed mf had completely been eradicated in these places.

7) Malayan Filariasis in Cheju Island, Korea:

Study on Malayan Filariasis in Cheju Island, Korea was performed jointly by universities of Nagasaki, Kagoshima and Seoul (Korea). Mf positive rate there was high (20.3%; 353 out of 1,714 examined). Mass treatment by DEC was done, with resultant side effects, especially higher fever onset than in cases of Bancroftian filariasis. The high fever was alleviated by steroid hormone administration. We have come to realize that due consideration is necessary on the occasion of international cooperation.

8) Pharmacodynamic Mechanism of DEC :

Microscopic study revealed that DEC affected the reproductive cell (sperm, ovum) of Dirofilaria immitis by inhibiting fertilization, which was confirmed by us.

Our study has centered mainly on filariasis. In the begining of our study, eradication of filariasis which had been endemic on detached islands and outlying districts was supposed to be impossible.

Long periods spanning 30 years have passed since then and I now feel I have accomplished my long-cherished purpose of filarial control. My profound gratitude is given to colleagues from Kagoshima University Hospital, Prefectural Government and people who cooperated with our filarial eradication project.

Last but not least, I can never thank late professor Sato enough for his kind instruction.

Special lecture

FUTURE TREND OF JAPANESE CONTRIBUTION TO THE INTERNATIONAL COLLABORATION IN MEDICAL SCIENCES

HIROYUKI HIRUMI
Research Center for Protozoan Molecular Biology Immunology, Obihiro University of Agriculture and Veterinary Medicine

Japanese contribution to the progress of international medical sciences may be divided into three categories: (I) development of modern technology, (II) medical aids in the states of international emergency, such as a natural disaster, a relief of famines and refugees etc., and (III) medical research and training in developing nations. Although the Japanese contributions to (I) and (II) have been progressively improving, a number of aspects in the area of (III) may require considerable improvements to achieve the efficiency of Japanese aids to developing countries. Firstly, prior to the initiation of any aid project in developing nations, it is essential to recognize the existence of great differences among such countries in their culture, levels of industrial and economical development and infrastructures. Therefore it is important to set up aid projects under a flexible aid policy which is highly compatible to the community of each recipient country. Secondarily the promotion of scientists who would be highly competitive to conduct medical aid projects in developing countries is urgently required. The present system in dispatching "Japanese Experts" to the aid projects would be one of the major factors that have been constricting the promotion of "Japanese Experts". The system which recruiting the experts by seconding scientists from universities and/or research organizations has been accounted for a number of demerits for the experts as well as for the institutions. The ability to recruit the experts under the present system, particularly in the medical research/training area, has been hitting the limit in Japan as a whole. A drastic change is therefor urgently needed in this area. To improve the problem it is highly recommendable (a) to found research/training centers of internationally high standard within existing medical institutions in several places in Japan, and (b) to establish "ODA Posts" within such centers under a novel system which is highly meritorious to the experts and the centers, aiming at a rapid progress in the promotion of the experts. Thirdly, the main objective of the medical aid projects should be diverted from the present "Technology Transfer" to "International Collaborative Research Projects" and execute "Problem Solving Joint Projects" with the collaboration of qualified local and Japanese scientists and in the future also with international experts. Objectives of the projects have to be focused on national problems of a high priority in the recipient country. Such projects should be completed within a term, not leaving "a ruin of ODA" benind. If the improvements suggested above would be achieved, Japanese aids in this area would become more efficient and make a greater contribution to the progress of medical sciences in developing nations in near future.

Symposium

STRATEGIES AGAINST TROPICAL INFECTIOUS DISEASES

YOSHIFUMI TAKEDA[1] AND ISAO TADA[2]
Research Center, International Medical Center of Japan[1]
and Department of Parasitology, Faculty of Medicine, Kyushu University[2]

In the control of infectious and parasitic diseases, various conventional treatment measures are becoming increasingly inadequate with the appearance of drug resistance in pathogens, insecticide resistance in vectors, lack of effective vaccines among others. Surveillance systems are usually badly functioning in many developing countries due to devastated economic and social conditions. Further, emerging new strains (*V. cholerae* 139) or species (HIV, HTLV, HCV) of virulent microorganisms are threatening humankind. These human pathogens are disseminating among the inhabitants abetted by the behavior and social customs widely practiced in tropical and subtropical regions. Tropical infectious and parasitic diseases are no longer a regional problem, but a global concern in the age of frequent international movement of refugee, migrant laborers and travelers. In this context, we designed this symposium to review desirable strategies against diseases caused by viral, bacterial and parasitic pathogens. Vaccine has undoubtedly top priority in the prevention of these diseases and certainly in the past it has conquered small pox successfully, although various important viral and bacterial pathogens still remain unchecked. No vaccines are available for parasitic infections. Individual pathogens have their own mechanism of regulating molecular and immunological identities. Intensive trials on the identification of specific targets in the pathogens will be necessary for breakthroughs.

Recently it has been rapidly recognized that anthropological approaches to the target communities are very important and necessary in the control of diseases. Without understanding knowledge, attitude and practice (KAP) of the community, it will not be possible to improve health level effectively. Thus, the combination of advanced technologies in biomedical science and active application of anthropological methodology should be encouraged in this era.

S—1

INSECT-BORNE VIRAL DISEASES

AKIRA IGARASHI
Department of Virology, Institute of Tropical Medicine,
Nagasaki University

Insect-borne viruses are members of arboviruses, many of which are human pathogens. Special attention has been paid for causative viruses of yellow fever (YF), Japanese encephalitis (JE), and dengue, because of the clinical severities, number of patients, and epidemic areas.

YF has been endemo-epidemic in tropical rain forests in Africa and South America. Although ideal vaccine has been developed, its effective application needs integration into national vaccination program especially EPI program.

JE has been prevalent in Asia monsoon areas, with large epidemics occurring in several developing countries in recent years. In order to overcome the high cost and limited supply of the current JE vaccine, WHO postulated to develop the second generation vaccine by modern biotechnology. Another approach is the local production of current JE vaccine and integration into EPI. JE in tropical Asia is an attracting research subject.

Dengue has been a world-wide health problem in the tropics, because of increasing number of patients, enlarging epidemic areas, and appearance of severe manifestation of dengue haemorrhagic fever (DHF). While dengue vaccine is still under development, and vector control could not provide long-lasting effect. Research on the vascular permeability factor and virulence gene of dengue viruses are challenging subjects.

S—2

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

NAOKI YAMAMOTO

Abstract: The etiologic agent of acquired immunodeficiency syndrome (AIDS) is a retrovirus called human immunodeficiency virus (HIV). The CD4 molecule on helper T cells serves as a receptor for HIV infection. The viral RNA is converted into DNA by its own reverse transcriptase and incorporated into cellular DNA, resulting in an establishment of persistent infection. In the presence of lymphocyte-activating factors or some other factors, HIV is activated *in vivo* in infected individuals, resulting in insufficiency of cell-mediated immunity. Indeed, it takes several years for infected individuals to develop clinical symptoms of AIDS after initial HIV infection. This clinical feature of AIDS suggests that some factors other than HIV itself may be related to the onset of AIDS. On the other hand, the rapid replication of HIV seems to directly affect the development or progress of AIDS. Therefore, anti-HIV drug therapy is also essential for AIDS. Most of the HIV-infected persons in Japan are hemophiliacs to whom HIV was transmitted by administration of clotting factors. What is required now is to protect these HIV carriers from the development of AIDS as well as to prevent further spread of HIV.

S—3

CHRONIC LIVER DISEASE IN SOUTH JAPAN: SPECIAL REFERENCE TO CHRONIC LIVER DISEASE X

TERUKATSU ARIMA
Second Department of Internal Medicine, Faculty of Medicine, Kagoshima University

In the early of the 1980 decade, prior to the studies that lead to the characterization of the HCV genome, we had the opportunity to treat successfully patients suffering from acute and chronic forms of non-A, non-B (NANB) hepatitis.

In 1989, my group cloned cDNA fragment, A-14 clone which is specific for NANB hepatitis. A-14 clone contains an epitope (AR142) recognized by the sera from HCV-infected patients and has 8/11 and 10/11 amino acid homology to the HCV-I and II, respectively. The epitope is localized between amino acid positions 8 to 18 of the core region of the HCV genome.

In addition, fifty-five clones encoding fragments of the HCV genome had been isolated by my group. The RNA and amino acid homologies between these clones and the prototype HCV, HCV-I, sequences ranged from 42% to 91%, and from 47% to 94%, respectively. The sequences covered by these clones corresponded to 60% of the total HCV genome. However cDNA corresponding to the M/E and NS2 regions were not obtained by our method. These results suggest that there is very low, if any at all, titer of antibodies against M/E and NS2 region in sera from patients with chronic NANB hepatitis, or from patients recovering from acute NANB hepatitis. In fact, recent studies have demonstrated high frequency of genetic mutations in the M/E, which may account for our observations and suggest a difficulty of development of the HCV vaccine.

Recently in 1993, 1,300 patients with liver diseases were visited our clinic. Liver biopsies were performed for 1,009 patients with liver diseases in the last 5 years. Twenty-three out of 90 patients with chronic active hepatitis type C were completely responded (CR) to an interferon treatment of 3MU, 3 times a week for 24 weeks and antibodies against A-14 peptides were considerably reduced in those patients with CR irrespective of the HCV type found in the patients.

In 1993, 43 patients with liver cirrhosis and 52 patients with primary liver cancer had visited our clinic. Thirteen (30%) and five (10%) of those cases, respectively, were of unknown (X) origin. Of these patients, 3 of which have chronic active hepatitis and 2 who have decompensated liver cirrhosis, all negative for HBsAg, anti-HCV antibodies, serum PCR-HCV negative, and also negative for HBV and HCV-PCR in the tissue have been studied extensively. All five patients presented

chronic active hepatitis which could not be controlled by corticosteroid treatment, although two of them presented plasma cell clustering in the portal area. These results suggest that these patients represent cases of CAH, liver cirrhosis and primary liver cancer due to new hepatotropic virus(es).

S—4

SEARCHING FOR THE ETIOLOGY OF HTLV-I ASSOCIATED MYELOPATHY (HAM)

MITSUHIRO OSAME, M.D.
The Third Department of Internal Medicine Faculty of Medicine Kagoshima University

The association of spastic paraparesis with human T-lymphotropic virus type I (HTLV-I) was demonstrated independently in two different areas; Caribian Basin[1] and Japan[2-3]. A serologic study in Martinique found that 59% of patients with tropical spastic paraparesis (TSP) had antibodies to HTLV-I.[1] Within several months another study showed the presence of antibodies to HTLV-I in both serum and cerebrospinal fluid (CSF) of TSP patients in Jamaica and Colombia. In Japan we also found an association of HTLV-I with spastic paraparesis, but because these patients reside in a temperate zone we proposed the term HTLV-I-associated myelopathy (HAM).[2-3] The finding of such patients in Japan suggested HTLV-I as a cause of spastic paraparesis because Japan is one of the most endemic areas in the world for adult T-cell leukemia-lymphoma (ATLL) caused by HTLV-I and is free from malnutrition and yaws, which had been considered possible causes of TSP. Some initial differences noted between TSP and HAM seem to have originated from the difference in historical background which led to the discovery of these conditions.[4] Other investigators have found a similar association between HTLV-I and myelopathy in other areas of the world, and now HAM and HTLV-I -associated TSP are thought to be the same disorder.[5]

A subgroup of cases with HAM has been identified, which was related to a previous history of blood transfusion.[6] The existence of another subgroup with mother -to-child transmission was also reported.[7] The identity of the viruses of HAM and ATLL was shown through DNA blotting[8] and DNA sequence analysis[9] from established cell line from CSF of HAM patient.[10] HLA -haplotype-linked high immune responsiveness against HTLV-I has also been reported.[11]

From October 1986 through January 1988, we conducted a nationwide survey of HTLV-I-associated myelopathy (HAM) in Japan to study its epidemiology, relationship to blood transfusion, and clinical aspects.[12] Four hundred and sixty cases were reported for a national prevalence of 0.35/100.000; the geographic distribution of HAM in Japan paralleled that of ATLL. Of the 460 patients, 26% had a history of blood transfusion, and the distribution of the interval from transfusion to onset of HAM followed a log-normal distribution. Significantly more transfusion-associated HAM cases were reported in HTLV-I nonendemic areas (37.6%) than in endemic areas (20.4%, $p=.001$) and among cases with no family history of HAM (28%) than in those with a family history (2%, $p=.001$). These results demonstrate an association between blood transfusion and the development of HAM and suggest that the transfusion of HTLV-I infected blood may load to the development of HAM. These data strongly suggest that the blood supply should be screened for HTLV-I before transfusion.

The mean age at onset of HAM was 41.2 years (range 5-75 years), and gait disturbance was the most common initial complaint. Onset of HAM was gradual in 92% of patients but the disease progressed more rapidly in older patients than in younger ones. Examination of cerebrospinal fluid frequently showed abnormalities and ATL-like cells were seen in the peripheral blood smear of 49% of patients. Favorable response to corticosteroid therapy was reported in 74% of patients.[13]

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S—5

DEVELOPMENT OF BACTERIAL VACCINE AND ITS FUTURE

HARUO WATANABE
Department of Bacteriology, National Institute of health

Several kinds of vaccines are now available in the world as listed below.

1) inactivated whole cell vaccines—*V. cholerae, B. pertussis*
2) live attnuatted vaccines—*S. typhi* Ty21a, BCG, *V. cholerae*
3) component vaccine—DPT, pili
4) polysaccharide vaccines—Hib, Vi-antigen, *S. pneumoniae, N. meningitidis*

Inactivated whole cell vaccines and polysaccharide vaccines tend to induce humoral immunity but not cellular immunity, while live attenuated vaccine induces cellular immunity too. Oral administration of vaccines can induce mucosal immunity. It is believed that mucosal and cellular immunity is much important than humoral immunity to prevent bacterial infection in the mucosa. So, one direction to develop future bacterial vaccines is to use live attenuated vaccines as a carrier for multivalent vaccine. In addition to the live attenuated vaccines, polysaccharide-protein conjugate is also one candidate as another future bacterial vaccines. In the below, multivalent vaccines now developing are described.

Multivalent vaccines

1) live attenuated vaccines(*S. typhi* Ty21a, BCG) expressing foreign antigens(tetanus toxin, HIV, *Pasmodium* etc.) or small peptides corresponding to foreign B- or T-cell epitopes.
2) polysaccharide-protein conjugate
   Surface polysacharide
   capsular polysaccharide: *H. influenzae* type b, *S. pneumococci, N. meningitidis* etc.
   carrier proteins:
   toxoids: DPT, LT, CT, VT etc.
   viruses: F protein of measles, protective antigens of varicella, rubella etc.

S—6

ERADICATION OF CHOLERA

HIDEO HAYASHI
Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba

Although more than one hundred years have passed since Koch identified *Vibrio cholerae* as a causative agent of cholera in 1882, the eradication of the disease or even the control of the organism have not been well established as yet. The major problem preventing us from the eradication may be as follows: 1. where and how *V. cholerae* survive or persist in natural environment? 2. how the organism aquire flexibility for mutation to survive under environmental stresses? and 3. why the vaccines so far evaluated by field trials failed to show high efficacy? The followings are some ideal strategies for the eradication of the disease.

The organism is unable to survive for a long period or to proliferate in the natural environment, strains isolated from environment sources do not have virulence genes like *ctx, ace, zot* etc., the organism proliferate only in human intestine and the root of infection is no other way but just fecal-oral mean by eating raw fish or vegetables like seashell, algea, seaweeds, or oyster, all of these evidence indicate that there should be a specific shelter or reservoir in our environment. We have to continue endless effort to identify the reservoir by applying advanced technology with deep insigt into the ecology of the vibrios.

The organism is provided with a magnificient capability to adapt to environmental stresses for their own survival. Such adaptational responce is initiated by recognizing the stress and then the signal is transduced

to a certain effector gene through the signal transduction system. The gene expressions are regulated by some upper ranking genes via sequential reactions, for instance, the production of the vilulence factors like *ctx, ace, zot,* and *tcp* are enhanced by *toxT* which is controled by *toxR* and *ToxS* as well as by the other signals from the environmental stresses. Physical, chemical or even biological stresses clearly induce many kinds of stress proteins in the organism, i.e. heat shock proteins, the roles of which have not been well characterized. These proteins should involve in the adaptational responce such as acquiring drug resistance or serotype conversion. The organism has been mutated from the calssical type to Eltor type through the past pandemics, although those were all 01 type, and recently, 0139 caused pandemic in the south west Asia of which population seemed to have been immunized by 01 type. In the endemic area, most of the isolates seemed to gain drug resistance, i.e. tetracycline resistance. It is evident that the organism gains a *de nove* property, most probably *via* mutation or transformation from the other organism under stressed condition and that the stress induced proteins including signal transduction components should play key role in the gaining process. If the sequential procedures are made clear at molecular level, then we can nail down which component is the key one to depress the production of vilurence factors or to repress the adaptational changes so as to keep the organism susceptible to certain drugs or to immunological defencing factors of human. We need to focus one of the research projects on this matter to obtain more detailed information and to create more effective strategy for the eradication.

Many cholera vaccines have been invented in US, Sweenden and/or Australia by applying gene technology and have being tested in field trials, but so far up to today, the efficacy has not been satisfactory. It is true that the convalescent patient acquirs vibriocidal humoral antibodies IgA, IgG and IgM which last for 3 months to one year. The invented vaccine were all effective as same level as the natural aquired immune. The involvement of memory T cell has been unclear, that is, molecular cascade reaction from antigen recognition and presentation through secretion of antibody or some ant-vibtio agents that prevent the organism from adhesion, proliferation, and/or production of the toxins should be clearly characterized.

The problems mentioned above should be investigated extensively at a well organized institution by efficiently organized scientists group supported by the national foudation.

S—7

MOLECULAR BIOLOGICAL STUDIES ON *ENTAMOEBA HISTOLYTICA*

YOSHIMASA KANEDA
Department of Infectious Diseases, Tokai University School of Medicine

It is estimated that 500 million people in the world are infected with *Entamoeba histolytica,* of whom 40 million have developed disabling colitis or extraintestinal abscesses, which will result in at least 40,000 deaths. However, more than 90% of infected individuals usually are asymptomatic. This is probably due to differences in the pathogenicity of amoebic strains. Although it is difficult to morphologically discriminate between pathogenic and non-pathogenic strains, it has been reported that biochemical and immunological differences exist. Sargeaunt and coworkers (1978, 1988) demonstrated that amoebic organisms isolated from patients with dysentery show isoenzymatic patterns that differ from those isolated from asymptomatic carriers. We reported that a monoclonal antibody, which recognizes the 30,000 Mr antigen, also can distinguish between pathogenic and non-pathogenic strains. However, zymodeme patterns and reactivities of monoclonal antibodies are phenotypic properties. Indeed, it has been reported that zymodeme conversion from nonpathogenic to pathogenic (or the reverse) can occur within a cloned culture of some strains of *E. histolytica* during the process of axenization under appropriate growth conditions. Therefore, molecular biological studies on the pathogenic properties of the amoeba are needed. The DNA sequence coding the 30,000 Mr antigen was detected not only in pathogenic isolates but in nonpathogenic isolates as well. However, in comparing both DNA sequences, minor differences were observed. In addition, we designed oligonucleotide primers on the basis of

the nucleotide sequence encoding the 30,000 Mr antigen, and have shown that the polymerase chain reaction technique using these primers is useful for determining pathogenicity. Numerous studies on the genetic analysis of pathogenic and nonpathogenic strains suggest that *E. histolytica* is a complex of two species, one pathogenic, the other nonpathogenic.

S—8

CONCEPTION FOR THE DEVELOPMENT OF SCHISTOSOMIASIS VACCINE

SOMEI KOJIMA
Department of Parasitology, Institute of Medical Science, The University of Tokyo

Schistosomiasis is widely distributed in developing countries and affects some two hundred million people, despite the fact that extensive efforts to control the disease have been continuously made in many endemic areas. Since the eradication of snail intermediate hosts is absolutely difficult and reinfection easily occurs among people living in the endemic areas even after a successful chemotherapy, the development of vaccines against schistosomiasis is urgently required. For this purpose, it is important to clarify effector mechanisms, on one hand, by which final hosts including humans can block the invasion of schistosomes or reduce the fecundity of the parasite in order to avoid severe damages of various tissues which result from immune responses of the hosts to deposited eggs. On the other hand, it is also necessary to clarify the targets of the effectors, such as that which molecule or which stage of schistosomes is critical in stimulating the effectors. In this symposium, recent observations including those of our own laboratory will be summarized and the important steps which should be taken into consideration in the development of schistosomiasis vaccine will be discussed.

S—9

MODIFICATION OF STRATEGY NEEDED FOR TROPICAL INFECTIOUS DISEASE CONTROL

HIDEO TAKEI
Faculty of Letters, Chiba University

Medical science has already accummulated sufficient knowledge and has developed efficient technology for tropical infectious disease control. And many Programs of tropical disease control have been launched without achieving planned and expected results. A case like the extinction of smallpox is rare. The task of efficient tropical disease control still remains as a hardest one to accomplish in most of the developing countries.

What was wrong with those Programs which had failed? Was technology employed inefficient? Financial support was poor? Or medical personnel involved, of those countries, lacked knowledge for the task or failed to learn it? Judging from reports presented here at this annual meeting, these conditions do not seem responsible for poor performance of the Programs.

The Programs of reeducating medical personnel, and reorganizing them for new, or more extended, tasks seem to have achieved fairly good results. A case of Tuberculosis Control Program presented here might be classified as this type.

On the contrary, however, when some Program entered directly into communities, with the intention to educate ordinary community members to change their daily habits, it met with unexpected obstacles, even when Health Education seems to have been successful. This was the case of Schistosomiasis Control Program in East Africa.

What makes the difference between these two cases? From the scientific point of view, i.e. seen as an applied practice based on the biomedical model of disease, both Programs are reasonable enough without any

doubt. A crucial difference between the two is the target groups, in the former case a group of medical personnel and in the latter ordinary community members. This fact suggests that the crucial factors in these cases are social, cultural and historical in nature.

Among various sociocultural factors, what should be taken into account, I argue, is the survival strategy and its underlying cosmology of the target groups. In the case of medical personnel, receiving further education, participating earnestly to acquire new skills, practicing newly acquired knowledge and skills, all these might fit into their survival strategy, because these can improve their competence as a salaried person. For the providers of the Program, medical personnel have another advantage. They are already educated to be able to share biomedical view point. It is not difficult to persuade them cooperate efficiently to fulfill their assigned task.

In the case of ordinary people, conditions are completely different. They do not share such view point, nor would be interested to learn and change their behaviour when there is not any other benefit to encourage their motivation. Moreover, they have their proper classification of diseases, which determines their priority for seeking medical assistance, and their survival strategy is based on it. When the target disease of a Program, more precisely, its signs and symptoms are culturally considered as benign ones, people will not change their behavior easily. Accepting what the Program recommends, sparing time for participating in its activities means, for them, putting asides for a while their daily activities, which would be of certain significance in their survival. They would never accept any change recommended by the Program personnel before analysing its merits and demerits. If the Program can not show any tangible merits according to their way of thinking, it is not strange that they consider it useless to listen to the advices of outsiders, who can survive without worrying about foods for today.

What is needed in formulating "New Strategy" for tropical infectious disease control is to understand people's survivan strategy, including the disease classification underlying it. Then, from the beginning of planning a Program, its planners and coordinators would be able to take such understanding into account for achieving better performance.

General Presentation

A—1

STUDIES ON PNEUMONIA AMONG THE CHILDREN IN BANGLADESH

YOSHIAKI UTSUNOMIYA[1], KAMRUDDIN AHMED[1], KIWAO WATANABE[1], MISAO TAO[1], GLENDA CAROL, MARTINEZ FRANCO[1], NAOTO RIKITOMI[1], TSUYOSHI NAGATAKE[1], KEIZO MATSUMOTO[1], SAMIR K. SAHA[2] AND RUHUL AMIN[2]
Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan[1] and Dhaka Shishu Hospital, Sher-e-Bangla Nagar, Dhaka, Bangladesh[2]

In Bangladesh pneumonia causes much morbidity and mortality among the children. The pathogen causing bacterial pneumonia among Bangladeshi children is not exactly known. We started this project in Dhaka Shishu Hospital, Dhaka, Bangladesh to know the exact pathogen of pneumonia by culturing pharyngeal and nasal swab. From May 1993 to October 1993 a total 157 cases were included in this study. There were 102 (65%) male and 55 (35%) female patients. The age ranged from 21 days to 11 years with the mean age of 14.4 months. Most of the patients came from the urban slum area. According to the nutritional status there were 117 (75%) of normal body weight, 14 (9%) of under weight, 14 (9%) of Kwashiorkor and 11 (7%) of Marasmus cases. There were 129/156 (82.7%) cases of bronchopneumonia and 27/156 (17.3%) cases of lobar pneumonia. Penicillins and gentamycin were administered in most of the patients. From the pharyngeal culture isolated bacteria were H. influenzae (16%), S. pneumoniae (14%), E. coli (11%), B. catarrhalis (8%), P. aeruginosa (8%), S. aureus (6%), E. cloacae (2%), K. ozanae (2%) and 23% isolated bacteria were mixed flora. From the nasopharyngeal culture isolated bacteria were B. catarrhalis (27%), H. influenzae (19%), S. aureus (16%), S. pneumoniae (6%), P. aeruginosa (6%), K. pneumoniae (6%), E. coli (3%), K. ozanae (3%), E. cloacae (1%) and 12% isolated bacteria were mixed flora. The isolated H. influenzae 76% were non-typable, 18% were type-b and 6% type e.

A—2

SURVEY OF PERTUSSIS IN GNANA

YASUHIKO KAMIYA[1], PATIENCE AKPEDONU[2], H.E.K. LONGMATEY[2], F.K. NKRUMAH[2], KIYOSU TANIGUCHI[2,3], HIROKI HORI[3] AND HITOSHI KAMIYA[4]
Department of Pediatrics, Shiga University of Medical Science[1], Noguchi Memorial Institute for Medical Research[2], Department of Padiatrics, Mie University School of Medicine[3] and National Mie Hospital[4]

A total of 56 clinical pertussis cases were identified in a sentinel survey at five polyclinics in Accra, Ghana for one year between August, 1992 and 1993 by using the case definition as 14 days of cough plus paroxysms of cough, whooping or vomiting. This active survey identified higer number of pertussis cases than passive surveillance data in Ministry of Health. By age, 51 cases were under five years old, including 13 under one year.

Of all 56 cases whose nasopharygeal swabs were collected and stored in Amies transport medium, nine cases yielded positive culture of *Bordetella pertussis* on Cyclodextrin Solid(CS) medium and/or Bordet-Gengou (BG) medium.

The isolation rate was 16.7% on each medium, though CS medium allowed smaller number of cells of *B. pertussis* to grow in experimental test.

In the nine positive cases, seven were under one year old and eight had not been three times or more immunized with DTP vaccines.

These findings show that pertussis vaccine was effective for the prevention of illness and should be administered fully as early as EPI schedule.

A—3

EVALUATION OF MURINE MODEL INFECTED BY *PENICILLIUM MARNEFFEI*

NORIFUMI KUDEKEN[1], NOBUCHIKA KUSANO[1], FUTOSHI HIGA[1], KAZUYOSHI KAWAKAMI[1], ATSUSHI SAITO[1], PRASIT THARVICHITKUL[2] AND THIRA SIRISANTHANA[3]
First Department of Internal Medicine, School of Medicine, University of the Ryukyus[1], Department of Bacteriology[2], Department of Internal Medicine[3] and School of Medicine, Chiang Mai University, Thailand

Penicillium marneffei, a dimorphic fungus that is endemic in southeast Asia, causes deep-seated infection in humans and rodents. Next to pulmonary tuberculosis and cryptococcal meningitis, disseminated infection with the *P. marneffei* is the common opportunistic infection in patients with the human immunodeficiency virus (HIV) in northern Thailand. We made murine model infected by *P. marneffei* and evaluated the effects of anti - CD4 antibodies on this model.

Specific pathogen-free BALB/c mice (male, weight; 20-25 g) were infected with intratracheal inoculation of the conidia of *P. marneffei* within the range of 3×10^6 to 1×10^8 cfu/mouse. The strain was *Penicillium marneffei* isolated from blood of patients with HIV. For the counts of viable *P. marneffei* of the organ, the homogenates prepared from the lungs, spleen and liver in diluted water with 0.1% Tweeen80 were cultured on Sabouraud dextrose agar. To deplete $CD4^+$ lymphocytes, mice were received intraperitoneal injection of anti-CD4 monoclonal antibodies (GK1.5).

After two weeks, the survival rate of mice with 1×10^8 cfu/mouse was 0%, 1×10^7 cfu/mouse was 50% and 3×10^6 cfu/mouse was 100%. On the histopathological examination, the lungs of infected mice showed infiltration of mononuclear cells and the destruction of alveolar structures at two weeks. After inoculation of 1×10^8 cfu/mouse, the viable *P. marneffei* were isolated from the lungs, spleen and liver on 3,7 and 10 days. The mortality of the mice depleted of CD4 lymphocytes is lower than that of control mice ($p<0.05$). The numbers of viable *P. marneffei* in the lungs and spleen of the mice depleted of $CD4^+$ lymphocytes were significantly increased ($p<0.001$) than that of the control mice at 13 days.

These results suggested that $CD4^+$ lymphocytes play the role in the destruction of alveolar structures by the infiltration of mononuclear cells and the removal of *P. marneffei* on this model.

A—4

EFFICACY OF CURRENT CLINICAL DIAGNOSIS OF ACUTE DIARRHOEAL DISEASES IN ZAMBIAN CHILDREN

NOBUYUKI MATSUBAYASHI[1], MASAHIRO WATANABE[1], G.J. BHAT[2], HITOSHI KAMIYA[1] AND MINORU SAKURAI[1]
Department of Paediatrics, Mie University School of Medicine[1] and Department of Paediatrics, University Teaching Hospital, Lusaka, Zambia[2]

INTRODUCTION: In most developing countries, acute diarrhoeal diseases are diagnosed as dysentery and non dysentery, according to macroscopic stool examination due to limited laboratory facilities. In this sense, dysentery might include diarrhoea caused by other type of pathogens. World Health Organization recommends adequate rehydration for both dysentery and non dysentery, and antibiotics only in exceptional cases such as bloody diarrhoea. In this study current clinical diagnosis was reviewed and evaluated.

METHOD: From May 1992 to May 1993, 690 of 3624 children with acute diarrhoeal diseases admitted to Diarrhoea Training Unit (DTU) of University Teaching Hospital, Lusaka, Zambia were recruited.

The inclusion criteria for this study were; 1. children less than six years. 2. History of ADD as defined by WHO (passage of two or more loose/watery stool in past 24 hours lasting less than 14 days) 3. No history of

prior antibiotic therapy.

Medical evaluation includes history by way of questionnaire noting clinical features of diarrhoea episode; physical examination noting degree of dehydration and concurrent diseases; laboratory work out including stool macro/microscopy, stool culture, polyvalent anti sera test for EPEC, ELISA kit test and electron microscopy for viruses.

RESULT: Among Dysentery cases, Shigella Dysenteriae was the first causative pathogen (23.2%) whilst EPEC was second causative pathogen (13.2%). On the other hand, Rotavirus was the major pathogen (28.2%) in Non Dysentery cases while EPEC was the second causative pathogen (15.8%). In terms of case fatality rate (CFR), EPEC had the highest percentage (15.8%), while both Shigellae and Rotavirus was about 9%. The group (EPEC infected cases both in Non Dysentery and Dysentery) on nalidixic acid which was found sensitive to EPEC had significantly lower CFR (0%) while those on other antibiotics (21.9%) and not on any antibiotics (14.3%) had higher CFR respectively.

DISCUSSION: The study reveals that EPEC was one of major causative pathogens of acute diarrhoeal diseases in Zambian children and has the highest CFR. Antibiotics treatment of acute diarrhoea with EPEC infection has not been established yet. However, treatment with nalidixic acid of the acute diarrhoeal diseases had significantly lower CFR in this study. We thus propose that not only dysentery cases but Non Dysentery cases as well with EPEC infection should be treated with appropriate sensitive antibiotics. Diagnostic procedure for easy detection of EPEC infection is necessary in the clinical evaluation of acute diarrhoeal diseases for prompt institution of appropriate treatment in order to reduce mortality.

A—5

BACTERIOLOGICAL INVESTIGATION ON THE DIARRHEAL DISEASES IN LA PAZ AND SUCRE, BOLIVIA IN 1993 AND 1994

AKIYOSHI UTSUNOMIYA[1,4], DANIEL ELÍO-CALVO[1], ARMANDO ALBERT BENITES REYES[1], ERNESTO SANZETENEA CASTRO[1], ENRIQUE RODRÍGUEZ[1], CAROLINA TORRES[2], JENNY I. ZAMORA DE CORZO[3], JUAN LUIS URÍA[1], ARNOLD HOFMAN-BANG SOLETO[1], AKEMI KAI[5] AND KAZUMICHI TAMURA[6]

Instituto de Gastroenterología Boliviano-Japonés La Paz[1], Sucre[2], Cochabamba[3], Bolivia; Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University, Japan[4]; Department of Microbiology, Tokyo Metropolitan Laboratory of Public Health, Japan[5] and Department of Bacteriology, National Institute of Health, Japan[6]

The aim of this study is to clarify the etiology and ecology of bacterial diarrheal diseases in La Paz (altitude 3,800 m with a thin atmosphere, chilly, low humidity) and in Surce (altitude 2,800 m), to contribute to the health of inhabitants.

Twelve hundred and thirty-four diarrheal rectal swabs were collected from outpatients and inpatients during the period between July 1993 and July 1994.

The numbers of isolated enteropathogens were as follows: 139 isolated strains (11.3%) for enteropathogenic *Escherichia coli* (EPEC), 80 (6.5%) for *Shigella*, 55 (4.4%) for enterotoxigenic *E. coli* (ETEC), 39 (3.2%) for *Salmonella*, 29 (2.4%) for *Vibrio cholerae*, 27 (2.2%) for enteroinvasive *E. coli* (EIEC) and 1 (0.1%) Verocytotoxin-producing or enterohemorrhagic *E. coli* (VTEC or EHEC), respectively.

In the age group 5 years and under (0-2 years plus 3-5 years), the isolation rate was 29.8%, and EPEC, *Shigella*, ETEC were also predominant. While in the age group 17 years and older, the isolation rate was 30.2%, and the predominant enteropathogens were EPEC, *V. cholerae*, *Salmonella*, ETEC and *Shigella*. In the age group between 6 and 16 years, the isolation rates were relativery low in each pathogen. Among EPEC isolates, O44, O55, O111 were predominant, and O6, O169, O153 for ETEC, O28ac was most common for EIEC. Among 10 strains of *E. coli* O157, only one strain produced Verocytotoxin (VT 2). Regarding the serovar of *Shigella*, *S. flexneri* 2a, 3a, and 1b were prodominant. In the case of *Salmonella*, S. Enteritidis was the most predominant, followed by S. Typhi, S. Poona and S. Paratyphi B. Out of 29 cholera cases, 25 strains belonged to serovar

Ogawa and remaining 4 were serovar Inaba.

The drug sensitivity tests were performed by disc and minimal inhibitory concentrations (MICs) methods using aminobenzil-penicilin (ABPC), sulfamethoxazole/trimetoprim (SXT), trimetoprim (TM), nalidixic acid (NA), gentamicin (GM), kanamycin (KM), tetracycline (TC), chloramphenicol (CP), erythromycin (EM), cefazolin (CEZ), ciprofloxacin (CIP), amikacin (AMK) and amoxicilin (AMPC). The resistant EPEC, ETEC, and *Shigella* strains against ABPC, SXT were most prominent.

In Bolivia, diarrheal patients are treated mainly with ABPC, or SXT, and rarely with GM, KM and others drugs. From these results, it is possible to say that ABPC and SXT are ineffective and invalid drugs for the treatment of bacterial diarrheal diseases, and resistant strains will increase and persist in the near future. While, NA showed high and KM, GM showed relatively high susceptible results in the sensitivity test. Therefore, it is very important to consider changing the antibiotics for the treatment of diarrheal diseases. NA, KM or GM are recomended for the available chemotherapeutants.

A—6

CONTROL OF CHILDREN'S BACTERIAL DIARRHOEA IN DEVELOPING COUNTRIES

TAKASHI NAKANO[1], HITOSHI KAMIYA[2], TAKESHI HONDA[3], HIROKI HORI[1], MASAHIRO WATANABE[1] AND NOBUYUKI MATSUBAYASHI[1]
Mie University School of Medicine[1], Mie National Hospital[2] and Research Institute for Microbial Disease, Osaka University[3]

Children's bacterial diarrhoea is one of the major health problems especially in developing countries. We conducted the survey of etiological agents and compared with clinical conditions in diarrhoeal children of African region. Moreover, we tried to clarify the source and mode of bacterial enteric infection. The main purpose of our study is to establish the effective control measures for children's infectious diarrhoea in developing countries.

We collaborated with three institutes of JICA (Japan International Cooperation Agency) projects in African region. They are Noguchi Memorial Institute for Medical Research (Ghana), Zambia University Teaching Hospital (Zambia) and Kenya Medical Research Institute (Kenya). Our study was also supported by the Grant for International Health Cooperation Research from the ministry of health and welfare, Japan.

The community-based study in Ghana (June 1987 -May 1988) showed that *Shigella* species were the most frequently isolated bacteria agents, followed by *Campylobacter*, EPEC and ETEC. The isolation rate of *Sh. flexneri* was significantly higher in diarrhoeal children. Although the difference was not statistically significant, the incidence of *Sh. dysenteriae* was similarly higher in the diarrhoeal group. *Shigella* species were also isolated to some extent from non-diarrhoeal children. The isolation rates of the other bacterial enteropathogen were not significantly higher in the diarrhoeal group. In formulating effective and practicable control measures, including antibiotic therapy, attention should be paid not only to children with diarrhoea but also the non-diarrhoeal ones.

The hospital-based studies in Ghana (September -November, 1992), Zambia (May 1992-May 1993) and Kenya (1993-) showed the most frequent isolates of EPEC followed by *Shigella, Salmonella* and *Campylobacter*. The severity of diarrhoeal illness didn't correlate with isolation rates of bacteria.

The bacteriological investigations for water source and home-kept water of diarrhoeal children were performed. The piped water and rain water were not contaminated, but the water from well and river was contaminated with bacteria. Several kinds of bacteria were isolated from home-kept water. Health education programmes such as 'handling of clean water' are also needed for the prevention of diarrhoeal diseases.

A—7

HUMAN ROTAVIRUSES ISOLATED IN CHAING MAI, THAILAND IN 1987-1993

TOMOKO URASAWA[1], KOKI TANIGUCHI[1], NOBUMICHI KOBAYASHI[1], KEIJI MISE[1], SHOZO URASAWA[1], AYAKO HASEGAWA[2] AND YUKIO YAMAZI[3]
Department of Hygiene and Epidemiology, School of Medicine, Sapporo Medical University[1], National Institute of Health, Japan[2] and Nippon Medical School[3]

Stool specimens were collected from pediatric patients with diarrhoea who were admitted to three hospitals in Chaing Mai (93% specimens were from McCormick hospital) during 6 consecutive years 1987 -1993. Four hundred fifty-seven stool specimens shown to contain group A rotaviruses by the demonstration of viral antigen by ELISA and / or of viral RNA in PAGE were subjected to this study. Ninety percent of the specimens were from those less than 24 months of age. Determination of subgroup and G (or VP7) serotype antigens of group A rotavirus was carried out by ELISA with subgroup (I and II)- and G serotype(1-4)-specific monoclonal antibodies or PCR with pairs of serotype -specific oligonucleotide primers.

Of 398 strains (87.1%) that could be subgrouped, 46. 8% were subgroup I, 37.0% were subgroup II, and 3.5% were subgroup I+II. Of 374 strains (81.8%) serotyped, 169 (37.0%) were G serotype 1, 179 (39.2%) G serotype 2, 8 G serotype 3, and 11 G serotype 4. Our 6 years' survey showed that the relative frequency of individual serotypes changed by year with dominant serotypes appearing in the order of G serotypes 1+2, G serotype 1 and G serotype 2.

Although almost all strains examined showed the usual correlation between subgroup, G serotype and RNA electropherotype found in human rotaviruses (i.e. subgroup I-G serotype 2-short RNA pattern, subgroup II-G serotype 1,3 or 4-long RNA pattern, or subgroup I+II-G serotype 3-long RNA pattern), seven strains having unusual characters were isolated. Strain Mc35 had subgroup I-G serotype 10-long RNA electropherotype, a property hitherto found in bovine rotaviruses. Strain Mc323, 345, 697 and 955 had subgroup I-serotype 9-long RNA electropherotype, and in RNA-RNA hybridization they were more related to porcine than to human rotaviruses. Mc930 and 1129 strains had G serotype 6 antigen that was usually found in bovine rotaviruses.

These results suggest interspecies transmission and genomic reassortment of animal rotaviruses in nature.

This study was supported in part by a Grant from Diarrheal Disease Control Program, WHO (Principal investigator: Y. Yamazi) and was carried out with collaboration from B. Pongprot, J. Supawadee and S. Suprasert, Chaing Mai University.

A—8

HEAT LABILE SPECIFIC ANTIGREN OF VIBRIO CHOLERAE O139

MASAAKI IWANAGA, MASAO TANABE, NOBORU NAKASONE, SATOSHI NAKAMURA, YASUKO HONMA, KAZUTOSHI NAKASHIMA, TOMOKO OWAN, TSUYOSHI SUNABE AND M. JOHN ALBERT[1]
Department of Bacteriology, Faculty of Medicine, University of the Ryukyus.
International Centre for Diarrheal Disease Research, Bangladesh[1]

It has been said that new cholera vibrio, Bengal, is almost the same with traditional cholera vibrios except nonagglutinability of the Bengal strains with O1 antiserum. However, there is a fact that post infection protective immunity of the traditional cholera is not crossreactive to the new cholera. Therefore, there must be some important differences in the antigenic substances between new and traditional cholera vibrios, as far as the sugar chain of LPS is not the protective antigen.

We have established a monoclonal antibody (MAb72) which clearly distinct Bengal strains from non -O139 Vibrio cholerae. The antigenic substance for

MAb72 was characterized so far as follows; 1) the antigen is located at cell surface as the agglutinogen of Bengal strains, 2) the antigen is easily inactivated by heating (100C, 1min), 3) the antigen is extracted by washing the cells, and the extract is reactive to MAb72 but not reactive after the treatment with SDS, 4) the extracted antigen does not pass through PM100 (100kDa cut) ultrafiltration membrane, 5) in gel filtration column chromatography using sephacryl S-1000, the antigen is eluted out before blue dextran (2,000 kDa), 6) there is a common epitope in the outer membrane protein OmpS, 7) the antigen is resistant to chroloform.

A—9

DISTRIBUTION OF GENES ENCODING CHOLERA TOXIN, ZONULA OCCLUDENS TOXIN, ACCESSORY CHOLERA TOXIN, AND ELTOR HEMOLYSIN IN *VIBRIO CHOLERAE* OF DIVERSE ORIGINS

HISAO KURAZONO[1], AMIT PAL[1,2], PRASANTA K. BAG[1,2], G. BALAKRISH NAIR[1,2], TADAHIRO KARASAWA[1], TATSUYA MIHARA[1] AND YOSHIFUMI TAKEDA[1,3]
Department of Microbiology, Faculty of Medicine, Kyoto University[1], National Institute of Cholera and Enteric Diseases, Calcutta, India[2] and Research Institute, International Medical Center of Japan[3]

A large collection of 1,154 strains of *Vibrio cholerae* of diverse origins including serogroups O1 and O139 and those belonging to the non-O1 and non-O139 (non-O1: non-O139) serogroups were examined with a battery of DNA probes specific for cholera toxin (CT), zonula occludens toxin (ZOT), accessory cholera toxin (ACE) and eltor hemolysin (HLY) to determine the distribution of genes among wild strains and to understand the importance of these factors in the pathogenesis of the disease cholera. Among the O1 clinical isolates, majority of the strains had genes comprising the core region intact and also possessed the HLY gene. Although rare, strains of O1 with natural deletions of the CT, ZOT and/or ACE genes were also detected. The combination of absence of the virulence genes comprising the core region but with the presence of the HLY gene dominanted among the O1 environment and food isolates and among the clinical and environmental non-O1:non-O139 strains of *V. cholerae*. All the O139 strains examined in this study possessed genes located in the core region and the HLY gene. There appears to be a natural attenuation of the O1 serogroup of *V. cholerae* as compared to the O139 serogroup. Among all the virulence-associated genes examined, the HLY gene was the most conserved genetic element in *V. cholerae* independent of biotypes and serogroups.

A—10

STRUCTURE AND FUNCTION OF RECOMBINANT PIG RECEPTOR (STaR) FOR A *Escherlihia coli.* HEAT-STABLE ENTEROTOXIN (STa) III: COMMON MOTIFS IN EXTRACELLULAR DOMAIN OF THE MEMBRANE-ASSOCIATED GUANYLYL CYCLASE RECEPTOR FAMILY INCLUDING HEAT-STABLE ENTEROTOXIN RECEPTOR (STaR)

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

Membrane-associated guanylyl cyclase (GC) receptors are a family of proteins which regulate intracellular cyclic GMP content in animal tissues. Several kinds of GC receptor have been reported: NPR-A(GC-A), NPR-B(GC-B), NPR-C, STaR(GC-C), retGC, and sapGC(APGC or SPGC). The structural features shared by these novel GC receptors include an extracellular hormone-binding domain, a single transmembrane

domain, and an intracellular signaling domain comprised of a kinase homology domain and a GC catalytic domain. The sequences of the extracellular domains are weakly homologous (17-43 % amino acid identity), whereas the intracellular domains are 36-78 % identical.

We compared the amino acid sequences of 13 GC receptors. For the homology search an algorithm for multiple sequence alignment was used. Six amino acid residues are highly conserved in the extracellular domain of all 13 membrane-associated guanylyl cyclase receptors [C^{72}, G^{98}, P^{99}, C^{101}, D^{347}, and D^{351} in pig heat -stable enterotoxin receptor (STaR)]. By considering common amino acids present, unique sequences were found including a GP motif ($CX_{20-25}GPXCXY$ and $CX_{20-25}GPXXXCXY$) and a DXXGD motif. To define the functional role of the DXXGD motif, each amino acid in the DNCGD sequence of pig STaR containing an Ha-epitope (Ha-STaR) was mutated to an Ala residue. Three Ha-STaR mutants which were substituted at conserved Asp347, Gly350, or Asp351 residue in the DXXGD motif did not retain STa-binding and GC-activities. Accordingly, the conserved amino acids of Asp, Gly, and Asp in the DXXGD motif of GC receptors are critical for conformational stability and/or activation of the GC catalytic domain.

A—11

EPIDEMIOLOGY OF HEPATITIS B VIRUS INFECTION IN ZAMBIA, AFRICA —THE IMPORTANCE OF HORIZONTAL TRANSMISSION IN CHILDHOOD AND RISK FACTORS—

HITOSHI OSHITANI[1], HRIOSHI SUZUKI[1], FRANCIS C. KASOLO[2], KATSUMI MIZUTA[2], MWILA MPABALWANI[2], NAKANDU P. LUO[2] AND YOSHIO NUMAZAKI[1]
Virus Research Center, Sendai National Hospital, Sendai[1]
and Virology Laboratory, University Teaching Hospital, Lusaka, Zambia[2]

It is known that sub-Saharan Africa is a high endemic area of hepatitis B virus (HBV) infection. However precise mechanism of HBV transmission is still to be defined, and control of HBV by vaccination has not been introduced in most of countries in Africa. An epidemiological study of HBV was carried out in Zambia as a part of Infectious Diseases Protect, a technical cooperation of Japanese Government, supported by Japan International Cooperation Agency (JICA).

[Material and Methods] 1. Normal pregnant women: a total 2,098 pregnant women attending to three (3) urban health centers in Lusaka and four (4) district hospitals in other provinces.

2. Children in urban community: a total 598 children aged 6 months to 15 years in urban community of Lusaka who were recruited by house to house hold survey.

3. Samples: serum was collected from pregnant women, and capillary blood was collected on filter paper from children.

4. Laboratory Methods: HBsAg-rPHA, HBeAg -rPHA, HBcAb-PHA and HBsAb-ELISA.

[Results] 1. An average of 6.5 % of pregnant women (137/2,098) was positive for HBsAg, and HBeAg was present in 16.1 % (22/137) of HBsAg positive sera.

2. Prevalence of HBsAg and antibody were significantly higher in rural areas than in Lusaka urban area.

3. Both HBsAg and antibody prevalence increased by age in children of urban community.

4. History of injection and blood transfusion were significant risk factors for HBV infection in children of urban community.

[Discussion] 1. It was shown that Zambia is a medium to high endemic area for HBV.

2. Horizontal transmission in childhood might be more important transmission route than vertical transmission.

3. Medical practices such as injection and blood transfusion were significantly correlated with prevalence of HBV markers.

4. There is a urgent need to establish cost effective control measure of HBV including prevention of risk factors.

A—12

SEROEPIDEMIOLOGICAL STUDY ON HIV, HBV AND HCV INFECTIONS IN CHIANG MAI, NORTHERN THAILAND

KAN TORIYAMA[1], AMNAT YOUSUKH[2] AND HIDEYO ITAKURA[1]
Department of Pathology, Institute of Tropical Medicine, Nagasaki University[1]
and Department of Pathology, Faculty of Medicine, Chiang Mai University, Thailand[2]

We performed a seroepidemiological study on the prevalence of hepatitis B(HBV) and hepatitis C virus (HCV) infections among the human immunodeficiency virus(HIV) antibody seropositive and seronegative blood donors in Chiang Mai, Thailand. The sera from 1,120 blood donors(male:952, female:168) were tested for anti-HIV antibody by using EIA(Abbott 3rd generation anti-HIV EIA). The positive sera were confirmed by using indirect ELISA(Anti-HIV, 1/2 Behring) and Gel Particle Aggulutination(GPA-anti-HIV, Kyowa Hakko). The anti-HCV antibody was assayed in all sera by 2nd generation EIA kit(Hepatostika C, Organon Teknika B.V.). The Hepatitis B surface Antigen(HBs-Ag) was tested by Reverse Hemagglutination(Thai Red Cross). HBs-Ag were detected in 20 out of 276 HIV seropositive blood donors(7.2%) and 68 out of 844 HIV seronegative blood donors (8.1%), respectively. HCV antibodies were detected in 17 out of 276 HIV antibody seropositive blood donors(6.2%) and 6 out of 844 HIV antibody seronegative blood donors(0.7%), respectively. These results suggest that anti-HIV antibody positive population belongs to a high risk group of HCV infection in northern Thailand.

A—13

SEROEPIDEMIOLOGY OF HEPATITIS VIRUSES A, B, C IN THE DOMINICAN REPUBLIC

A. SHICHIJO[1], H. AONO, H. SHIBAYAMA, H. TERAO, A. YIYATA,
H. OZAWA, M. ITOU, J. MISURI, K. MIFUNE AND T. ITOGA
Department of Microbiology, Oita Medical University[1]

First, 408 sera (Group I) selected at random from the sera collected at the Department of Clinical Laboratory of the Center of Gastroenterology, Aybar Hospital, Dominican Republic were examined for the prevalence of hepatitis A virus (HAV) antibody, hepatitis B virus (HBV) antigen (HBsAg) and hepatitis C virus (HCV) antibody, and secondly, 2,000 healthy sera (Group II) collected at various 6 regions of the country were examined for HBV antibody and HBsAg. (1) HAV antibody positive rate was less than 70% in age groups of 6 months to 10 years and reached 97% in a age group of 15 years old and 100% in age groups of more than 20 years. This result is almost identical to those in many countries of Southeast Asia and Africa. Because the prevalence of anti-HAV has been shown to be the index of hygienic and sanitary conditions, futher studies are necessary to observe the improvement of hygienic conditions in the future. (2) The overall prevalence of HBsAg among Group I was 4.7%. The rate was significantly higher in males (6.3%) than that in females (1.5%) ($P<0.05$). The overall prevalence of HBsAg among Group II was 3.2%. The rate was also significantly higher in males (4.8%) than that in females (2.0%) like Group I ($P<0.05$). The overall prevalence of anti-HBs was 17.7% but no significant difference was observed in those between males (20.0%) and females (16.1%). According to the classification by WHO, the above rate in this country was intermediate prevalence region type (HBs antigen positive rate, 2-7%) and was higher than that in Japan. (3) HCV antibody positive rate was examined by using the 2nd generation recombinant ELISA kit. The overall prevalence of anti-HCV was 4.7%, indicating that the rate is markedly higher when compared with those in Americas and Europe (0.2-1.4%) and in Japan (1.0%). This positive rate did not differ significantly between the males (4.9%) and the females (4.5%). Anti-HCV was found only in a small number of individuals in age groups of up to 40, but showed abrupt increases in

age groups of above 50 : 10, 20, and 22% at age groups of 51-55, 56-60 and above 61, respectively. In the present study, however, the reason why higher positive rates were observed in higer age groups could not be elucidated.

The present study is a joint one with A. Hidalgo, M. B. Castro, L.V. Sora, A. German and E.N. Maria (Center of Gastroenterology, Aybar Hospital, Dominican Republic)

A—15

AN EFFECTIVE COMBINATION THERAPY WITH ORAL FLUCONAZOLE (FLCZ) AND FLUCYTOSINE (5-FC) AGAINST CRYPTOCOCCAL MENINGITIS (CM) IN AIDS PATIENTS IN UGANDA

KAZUNORI OISHI[1], SATOSHI MITARAI[1], HIROSHI YAMASHITA[1], KIWAO WATANABE[1], TSUYOSHI NAGATAKE[1], ROY MUGERWA[2] AND KEIZO MATSUMOTO[1]
Dept of Int Med, Inst of Trop Med, Nagasaki Univ[1] and Dept of Med, Makerere Univ, Uganda[2]

We evaluated a clinical efficacy of anti-fungal chemotherapy with FLCZ and 5-FC for CM in AIDS patients in Uganda. This study was designed to compare the clinical efficacy of FLCZ and 5-FC for first 2 weeks with that of FLCZ alone. In this study, we employed a dose of 200 mg for FLCZ, because the clinical efficacy of FLCZ at 200mg was comparable to that of FLCZ at a 400mg in the previous study. 57 patients were enrolled for this study. These patients receiving each regimen showed the similar mean age (34 y.o.), male sex (35-48%), and incidence of oral candidiasis (60-79%). No significant difference was shown in the CD4 levels (73-81/mm^3) or CD4/8 ratios (0.41) before treatment between two groups.

We could evaluate survival rate of 46 patients at the end of primary therapy for 2 months. Although the group receiving FLCZ alone showed a survival rate of 35%, the group receiving FLCZ+5-FC prevented the early death within 2 weeks and significantly increased the survival rate (61%; $P<0.05$). No serious adverse events of these antifungal drugs was obaserved in the both group. We also compared CD4 levels of patients in each treatment group between before and after primary therapy. We found a similar decrease of CD4 levels in both treatment group, but a statistical significance was demonstrated only in the group receiving the combination therapy. CD4 levels in peripheral blood appears to decline with the progression of HIV infection despite an effective anti-fungal chemotherapy.

In this paper, we demonstrated an efficacy of combination therapy with FLCZ at a dose of 200mg and 5-FC for AIDS patients with CM in Uganda. This combination therapy may prevent the early death of these patients, and also provide a higher survival rate at the end of treatment and prophylaxis for 6 months.

A—16

BACTERIAL MENINGITIS AMONG BANGLADESHI CHILDREN ATTENDING IN A CITY HOSPITAL

KAMRUDDIN AHMED[1], YOSHIAKI UTSUNOMIYA[1], SAMIR K. SAHA[2], KIWAO WATANAGE[1], RUHUL AMIN[2], GLENDA CAROL MARTINEZ FRANCO[1], TSUYOSHI NAGATAKE[1] AND KEIZO MATSUMOTO[1]
Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan[1] and Dhaka Shishu Hospital, Sher-e-Bangla Nagar, Dhaka, Bangladesh[2]

Bacterial meningitis causes much morbidity and mortality among the children of Bangladesh. The real incidence and prevalence of meningitis is still unknown. Since 1993 we started to study bacterial meningitis

among children attending Dhaka Shishu Hospital, Dhaka, Bangladesh, a hospital only for children. Until now 103 cases with suspected bacterial meningitis has been studied. The male and female cases were 60 and 43 respectively. The age ranged from 6 days to 12 years old children. In this study cerebrospinal fluid (CSF), blood and swab from the nasopharynx were cultured at the same time. There was bacterial growth in 61 specimens of CSF. The isolated bacteria were *H. influenzae* in 34 cases, *S. pneumoniae* in 13 cases, *N. meningitis* in 8 cases, Gram negative bacilli in 3 cases, *E. coli* in 1 case, *V. metschnikovii* in 1 case and *F. meningosepticum* in 1 case. In all cases of meningitis single pathogen was isolated. Among 7 cases same species of bacteria were also isolated from the blood. Only in one case *H. influenzae* was isolated from nasopharynx, CSF and blood culture. All strains of *H. influenzae* were typable. 85.4% were of type b and 14.6% were non-b-type of *H. influenzae*. We suggest the introduction of vaccine against *H. influenzae* might decrease the incidence of meningitis among the children of Bangladesh. The high risk group in meningitis cases were (i) patients with lower socioeconomic status (ii) meningitis by *H. influenzae* and (iii) patients treated with penicillin plus chloramphenicol. Further studies are progressing to identify the serotypes of *S. pneumoniae*.

A—17

ACTIVITY AND THERAPEUTIC EFFICACY OF ANTIBIOTICS AGAINST *BURKHOLDERIA PSEUDOMALLEI* AND ITS PNEUMONIA IN MICE

HIROSHI FUKUHARA[1], FUTOSHI HIGA[1], ATSUSHI SAITO[1], NOBUTIKA KUSANO[2], PARASIT THRAVICHITKUL[3] AND CHARLIE PHORNPHUTKUL[3]
First Department of Internal Medicine, University of Ryukyus[1], Central clinical Labotatory, University of the Ryukyus Hospital[2] and Chiang Mai University, Thailand[3]

Melioidosis is an important infectious disease with a high morbidity and mortality rate in tropical areas. Recently, melioidosis has been carried elsewhere by workers or travellers from an endemic area.

We confirmed *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*) to be the causative pathogen of melioidosis *in vitro* and *in vivo*. 13 strains of *B. pseudomallei* isolated from Thailand showed resistance to a variety of drugs such as ampicillin, cefazolin, cefotiam, gentamycin, erthromycin, and clindamycin. Imipenem showed the highest antibacterial activity as measured by its MIC, but a 1×MBC(4×MIC) dose of imipenem did not inhibit intracellular growth of macrophages. In contrast a 1×MBC(2×MIC) of levofloxacin inhibited intracellular growth of macrophages despite of lesser MIC than imipenem.

Levofloxacin(10mg/kg) was significantly more effective than ceftazidime(100mg/kg), imipenem/cilastatin(50mg/kg), piperacillin(100mg/kg) and minocycline(10mg/kg) against *B. pseudomallei* pneumonia in mice. Imipenem/cilastatin and ceftazidime were almost equally effective against the infection but the required dosage for imipenem/cilastatin was half that of ceftazidime. Acridine orange stained preparations of lungs of surviving mice not administered any antimicrobial agent were observed for intracellular viability of multiplied bacteria through fluorescence microscopy 72 hr after infection.

Levofloxacin may have a useful clinical role in treating melioidosis caused by *B. pseudomallei* to be facultative intracellular growth of organism in human such as the genera *Salmonella, Shigella* and *Listeria*.

A—18

VIROLOGICAL STUDY OF DENGUE EPIDEMIC IN VIENTIANE, LAO P.D.R. IN 1994

THONGCHANH SISOUK[1], KAZUMI KANEMURA[2], MIKA SAITO[1,2,4], BOUNLAY PHOMMASACK[3], YOSHIHIRO MAKINO[2], SAKAE ARAKAKI[2], TOSHIHIKO FUKUNAGA[2] AND SITHAT INSISIENGMAY[1]
National Institute of Hygiene and Epidemiology, Lao P.D.R.[1],
Department of Virology, Faculty of Medicine, University of the Ryukyus[2],
Vientiane Municipality Health Service, Lao P.D.R.[3] and JICA Lao PHC Project[4]

In 1994, dengue (DEN) epidemic occurred after a silent interval of 7 years in Vientiane Municipality. By 17th of October, a total number of 1590 dengue fever and dengue hemorrhagic fever (DF/DHF) cases were reported. The outbreak was first reported in May from Ban Hom commune, in a rural district, about 15 km southeast from the center of Vientiane. On 1st of June, a survey was conducted at this commune. A total of 107 serum specimens were collected from the children under 16 years-old who had fever within 5 days were tested for virus isolation using cultured mosquito cells (C6/36). Sixteen dengue virus strains were isolated from 15 serum specimens. The positive rate of virus isolation was 41.8% on the second day and 21.1% on the third day of illness. Double infection with DEN-1 and DEN-2 was found in one case with type-specific monoclonal antibodies and further confirmed by RT-PCR. All serum specimens were tested for DEN-specific IgM by IgM-capture ELISA. The IgM antibody could be detected on the second day and the positive rate increased thereafter and reached 60% on the fifth day of illness.

A—19

DF/DHF EPIDEMICS AND CONTROL IN VIENTIANE MUNICIPALITY, LAO P.D.R. IN 1994

MIKA SAITO[1,5,6], BOUNLAY PHOMMASACK[2], KHAMPE PHONGSAVATH[3], BOUNLEUA PHILAVONG[4], DUANGDAO SUKHALUNG[4], TRAYKHOUANE PHOUTAVONG[5], SITHAT INSISIENGMAY[5], S. SALAMANY[7], YOSHIHIRO MAKINO[1], TOSHIHIKO FUKUNAGA[1] AND KIYOSHI SUZUKI[6]
Department of Virology, Faculty of Medicine, University of the Ryukyus[1], Vientiane Municipality Health Service[2], Sethathirath Hospital[3], Mahosot Hospital[4], National Institute of Hygiene and Epidemiology[5], JICA Lao PHC project[6] and Ministry of Public Health Lao P.D.R.[7]

Dengue fever and dengue hemorrhagic fever (DF/DHF) is one of the most important public health problems in Lao P.D.R. In Vientiane Municipality, the capital of Lao P.D.R., Aedes Control Unit (ACU) has been organized and conducted dengue control activities in 4 urban districts which include larval survey, health education and insecticide spray, to reduce the larvae density. In the beginning of 1994, Dengue Control Network was set up to strengthen surveillance system, laboratory capability and health education, and to make an effective response to the situation. In 1994, epidemic of DF/DHF occurred in Vientiane Municipality after an interval of 7 years, and a total of 1590 cases with 5 deaths were reported by 17, October, 1994. The outbreak occurred in 4 urban districts and 2 rural districts where the ACU had not conducted its activity. The morbidity rates of the rural districts were higher than those of the urban districts. No epidemic was reported in another 2 adjacent rural districts. At Ban Hom commune (population is 968), which belongs the rural district, 17 and 23 cases were reported in May and June, respectively. The larvae density was high (Breteau Index was 188) at the end of May, 1994. On receiving information of dengue outbreak through Dengue Control Network, health education, larval survey, larviciding and mass fogging of insecticide were carried out immediately. In August, the Breteau Index and the reported cases in this commune decreased to 7.9 and 0, respectively. From these observation, it is recommended that the dengue control should be more strengthened in

Vientiane Municipality. (We are grateful to Dr. Senkham Boudala for his assistance)

A—20

EVALUATION OF FILTER PAPER METHOD FOR THE COLLECTION AND STORAGE OF BLOOD AND SERUM SPECIMENS IN THE ASSAY OF ANTI-DENGUE IGM-ELISA

AKIRA IGARASHI[1], KOUICHI MORITA[1], SUNTHAREE ROJANASUPHOT[2], SUDARAT RUANGTRAKIT[2], SUMLEE DUANCHANDA[2], AREERAT SRIJUGGRAVALOVONG[2], SOMYOS KANUNGKIT[3], PICHAI THONGTRADOL[3] AND PISIT NIMNAKORN[3]

Department of Virology, Institute of Tropical Medicine, Nagasaki University[1],
Virus Research Institute, National Institute of Health, Thailand[2]
and Nakorn Phanom Provincial Hospital, Thailand[3]

Dengue virus infections have been prevalent in the tropics worldwide, and is counted among the leading cause of hospitalization and death among children in southeast Asia. Laboratory diagnosis on dengue has most frequently been carried out by serology, using classical hemagglutination-inhibition (HI) tests. Recently, IgM-ELISA was introduced into dengue serology to improve diagnostic efficiency. Because of the convenience, most of the specimens for dengue serology have most been collected, stored and shipped as filter paper dried blood. Although the suitability of filter paper method was assessed for the HI test, it has not been critically evaluated for the IgM-ELISA.

During June to July 1993, 135 hospitalized dengue patients were bled for their acute and convalescent specimens. A part of the specimens was directly dried on filter paper (Advantec No. 1) for storage at room temperature for 1, 3, 4, and 5 months. Serum was separated from each of the remaining specimens and a portion of it was dried on the filter paper and stored as above. The remaining serum specimens were aliquoted and stored at −20°C.

IgM-capture ELISA was carried out according to the AFRIMS method on (1) aliquoted serum, (2) filter paper dried blood and (3) filter paper dried serum specimens, in parallel after storage period of 1, 3, 4, and 5 months. The results on (1) aliquoted and frozen specimens showed high reproducibility, both in terms of diagnostic results and ELISA titer. The IgM-ELISA on filter paper dried specimens, on the other hand, showed decreasing numbers of primary dengue cases according to the storage period, while the number of secondary dengue cases did not change so much. The IgM-ELISA titer of filter paper dried specimen also showed a decreasing trend as the storage period was prolonged. The decrease was more pronounced when serum was dried rather than the whole blood, resulting in the decreased titer for secondary cases also.

The results showed that when the filter paper method was used for sample collection, storage and shipment, whole blood instead of serum should be used and the IgM-ELISA should be carried out within one month from the sample collection. Otherwise there will be false negative results for primary dengue cases. Differential stability of anti-dengue IgM ELISA antibody titers in primary and secondary dengue cases indicated some qualitative difference between the IgM antibodies produced in the primary and secondary dengue infections.

A—21

A NEW DNA ALLELE OF HLA-B IN ORANG ASLI POPULATION IN MAINLAND OF MALAYSIA

MOHD. ZAIDI ABU SAMAH[1], KENJI HIRAYAMA[2], NASURUDDIN HJ. ABDULLAH[1], ONG KIAN JOO[1], LOKMAN HAKIM[3], MAK JOON WAH[3], KATUSI TOKUNAGA[4], LIN LING[4], HIROSI TANAKA[3], SOMEI KOJIMA[5], MIHOKO KIKUCHI[2] AND EITARO HORI[2]
Institute of Medical Research, Malaysia[1], Department of Medical Zoology, Saitama Medical School[2], JICA-IMRTropical Disease Project[3], Japan Red Cross Blood Center[4], Medical Research Institute, University of Tokyo[5]

To analyze the genetic factors influencing the susceptibility or resistance to Malaria in orang asli population, we have typed for their HLA-B using DNA typing (sequence-specific oligonucleotide probes: SSO method) and conventional serology. 114 blood samples from Bentong Village and 58 from Gombak hospital were obtained to analyze.

We found one specific type that has a unique sequence of Exon 2 of HLA-B containing at least one recombination between Bw75.1 and Bw4. The antigen frequency of this type was estimated to be around 40%. Serologically, it was typed for B62 with Bw4.

Using SSOP method here revealed amino acid sequence that makes pockets fitting to antigenic peptides. When compared with B53 sequence that has been reported to be resistant type against malaria in Gambia, p9 pocket of B77 was completely similar to that of B53. Therefore, it was suggested that the same infectious agent functioned as an important evolutional pressure on HLA genes. Determining the whole sequence of this type is under investigation.

A—22

STRUCTURAL ANALYSIS OF POPULATION PROBLEM IN VIETNAM

SHINYA MATSUDA
Department of Preventive Medicine and Community Health, University of Occupational and Environmental Health, Japan

Structure of population problem in Vietnam was analyzed based on a fertility model developed by the Committee on Population and Demography. In this investigation, I employed the correlation analysis and the regression analysis in order to clarify factors associated with fertility, based on the following data: 1989 census data, 1989 statistical yearbook, results of questionnaire distributed in the communities. According to the results, the most important factors associated with the fertility level in Vietnam are the literacy rate of women in reproductive age, the proportion married among the female in reproductive age, and the infant and early childhood mortality rate of the region. In addition to these factors, the availability of health facility also showed a statistically significant correlation with the fertility level.

It is predicted that the fertility level in Vietnam will decrease steadily because of the change of the reproductive behavior accompanied with a rapid economic development and the urbanization. However, this process will result in a number of social pathology, i.e., street children, abandoned aged, increase of the crime, etc. In order to realize a sound economic development, they have to implement some integrated program, especially a special consideration is necessary for the education and the employment of women and minority. Furthermore, the development of a social supporting system for the elderly must be prepared from now in order to cope with a coming aging society resulted in the rapid decrease of the fertility. It is expected to implement some international cooperative programs between Vietnam and Japan.

A—23

MECHANISM OF EOSINOPHIL ACCUMULATION IN CEREBROSPINAL FLUID OF *ANGIOSTRONGYLUS CANTONENSIS* INFECTED MICE

KENTARO YOSHIMURA, HIROKO SUGAYA AND KAZUTO ISHIDA
Department of Parasitology, Akita University School of Medicine

In *Angiostrongylus cantonensis* infection, cerebrospinal fluid (CSF) eosinophilia is provoked in nonpermissive hosts (mice, guinea pigs and humans) but not in permissive rat host, and furthermore, infected nude mice fail to induce CSF eosinophilia (Sugaya & Yoshimura, 1988). In an attempt to determine the mechanism(s) of CSF eosinophilia in nonpermissive hosts, therefore, *A. cantonensis* infected nude mice (AF-nu/nu) were iv transferred with CSF eosinophils harvested from donor AF-nu/+ mice previously infected with the same parasite, and then examined for CSF eosinophilia. This study revealed that CSF eosinophilia could be provoked in nude mice infected with *A. cantonensis* for 17-20 days but not in uninfected normal nude mice, both of which were transferred with CSF eosinophils (eosinophil purity = 90%) harvested from infected donor nu/+ mice, suggesting that the presence of *A. cantonensis* worms in the brain was essential for the induction of CSF eosinophilia. In order to determine whether the source of eosinophils to be transferred affect the results of eosinophil accumulation in recipient CSF, intraperitoneal eosinophils (eosinophil purity = 77%) from donor nu/+ mice previously infected with *Mesocestoides corti* were iv transferred to nude mice infected with *A. cantonensis* for 17 days. This study demonstrated that the nude mice transferred even with the intraperitoneal eosinophils were capable of inducing significant CSF eosinophilia. Moreover, *A. cantonensis* infected nude mice were iv transferred with eosinophils which had been labeled *in vitro* with a Zynaxis' PHK-26 fluorescent staining kit. This experiment showed that almost all of eosinophils accumulated in the recipient CSF were labeled ones, suggesting that eosinophils accumulated in CSF were transferred eosinophils themselves, but not eosinophils newly produced by the recipient mice.

An additional experiment was conducted to determine whether eosinophil chemotactic factor(s) (ECF) derived from young adult worms (YA) are involved in eosinophil accumulation in the mouse CSF; two kinds of monoclonal antibodies against ECF-YA (Ishida & Yoshimura, 1992) were mixed and then, a total of 8 mg of the mixed antibody, dividing into four injections, was given to recipient nude mice before and after eosinophil transfer. This antibody treatment, however, failed to block CSF eosinophilia in the recipient mice. The most important finding in the current study was that *A. cantonensis* infected nude mice iv transferred with CSF eosinophils yielded significantly lower worm recovery than those transferred with medium alone, suggesting that newly transferred eosinophils directly migrated to CSF and played an important role in killing intracranial worms.

A—24

CLINICAL STUDY ON HTLV-1 INFECTION AND STRONGYLOIDIASIS

OSAMU ZAHA[1], NAGISA KINJO[1], TAKESHI UEHARA[1], AKIHIKO KOUCHI[1], JUNICHI OSHIRO[1], KOUSHIN SIKIYA[1], FUKUNORI KINJO[1,2] AND ATSUSI SAITOU[1,2]
1st Department of Internal Medicine[1] and Research Center of Comprehensive Medicine[2], Faculty of Medicine, University of The Ryukyus

Strongyloides stercoralis infection and anti-HTLV-1 antibody have been examined in patients in hospitalized since July 1988 to August 1994.

There were 2 cases with hyperinfection syndrome, one disseminated strongyloidiasis with meningitis and 115 asymptomatic cases. Strongyloides stercoralis

infections were 12.3% in male and 5.9% in female.

Anti HTLV-1 positive rate were 17.9% in male and 23.9% in female. Strongyloides stercoralis infections were 22.5% in male and 11.1% in female who were cases with HTLV-1 infection, which were statistically higher than cases without HTLV-1 infection.

Two hundred ninty-five patients with malignant neoplastic leision, respiratory or digestive organs were investigated in this study, which revealed the patients with Strongyloides stercoralis infection has been highly complicated by malignancy than patients without Strong yloides stercoralis infection.

A—25

EPIDEMIOLOGY AND CONTROL OF MALARIA IN YUNNAN PROVINCE, PEOPLE'S REPUBLIC OF CHINA

CHE LI-GANG, DIRECTOR
The Research Institute of Malaria Prevention
and Treatment of Yunnan Province

Several disasterous malaria epidemics in the Yunnan Province were recorded in the past. The railway construction scheme started in 1901 caused a epidemic prevailed in the region with 200,000 recorded deaths. In 1919, another malaria epidemic occurred in Simao where only 1000 people out of 70,000 total population could escape from the infection. In the epidemics occurred in 1933, 30,000 casualities were resulted. A systematic malaria control programme was set out in 1956 and had been continued successfully. In 1954, annual parasite incidence(API) was 234(/10,000); the rate decreased sharply to 11.08 in 1965. Recorded death was 1102 in 1954, while, only 6 death was reported in 1965. This success was interrupted by a political disorder, and in 1973, API peaked at 56.18 with 271 deaths. After overcoming the trouble, in 1993, API of total Province was lowered to 4.01. However, dead cases in the same year were 15. The present control efforts are concentrated along the borderline zone where the chloroquine resistant falciparum malaria makes serious problem. Also, population movement causes a difficulty in the control operation. Research activity so far, studies on the risks of global warming on malaria epidemics are being worked with Japanese experts.

A—26

MALARIA EPIDEMIOLOGY IN LOMBOK ISLAND, INDONESIA; APPLICATION OF SERO-EPIDEMIOLOGICAL METHODS

YOES P. DACHLAN[1], SOEDARTO[1], SRI HIDAJATI B.S.[1], BARIAH IDEHAN[1], KUSMARTISNAWATI[1], ANI SAFRIAH[1], MACHFUDZ[1], SUBAGYO YOTOPRANOTO[1], SRI SUBEKTI[1], EDDY SOEWANDOJO[1], PARWATI S.B.[1] ET AL., MARTONO[2], BAMBANG WIRATMADI[2], KUNTORO[2], AGUS SUTANTO[3], UAENAL ARIFIN[3] ET AL., SUBAKTININSIH[4], TATONG HARIYANTO[4], K. TANABE[5], I. MIYAGI[5], S. UGA[5], F. KAWAMOTO[5] AND H. KANBARA[5]
Airlangga University, Surabaya[1], Department of Health, Province of East Jawa[2],
Department of Health, Province of Nusa Tenggara Barat[3], University of Braijaya[4] and Adviser[5]

Based on the preliminary epidemiological survey of malaria in the Lombok island, Indonesia in 1991, we selected three subvillages and conducted the regular malaria survey at intervals of two months from August, 1992 to June, 1993. In the survey the sero-epidemiological methods using CS and RESA repeat peptides were introduced and evaluated for their applicability. As reported previously antibodies to CS repeats were short-lived, and consequently were useful to determine the epidemic season. Two subvillages showed the similar epidemic pattern, namely two peaks of seropositive rate on August and April which were

supposed to be caused by *Anopheles* spicies breeding in brackish water along the seaside such as lagoons, rivers and fish ponds. On the other hand the other subvillage showed two separate epidemic patterns, one was similar to that in the former two subvillages but the other was distinct from it. This distinct epidemic was found on a hilly area 3-4 km apart from the sea and showed two lower peaks of seropositive rate on October and June. Causative *Anopheles* species remain to be determined.

A—27

A CASE OF ACCIDENTAL TRANSMISSION OF *PLASMODIUM FALCIPARUM* THROUGH PLATELET TRANSFUSION

SHIGEYUKI KANO AND MAMORU SUZUKI
Department of Parasitology, Gunma University School of Medicine

The patient was a Japanese female, 70 years of age, resident of Funabashi, Chiba, and had never been abroad. The woman suffered from thrombocytopenia and received a total of 60 units of platelet concentrate during the period, 4-13 April, 1991. On April 24, she manifested a fever of 39°C which was successfully treated with Voltaren Suppo™, a non-steroidal anti-inflammatory drug. Her body temperature remained normal until May 1, when she suddenly developed symptoms of cerebral malaria, liver and renal dysfunctions. In the early morning of May 2, she lost consciousness and went into cardiac arrest. Giemsa stained thin blood smears obtained for blood cytological examination which were kept at the laboratory of the hospital, later revealed that she had contracted *Plasmodium falciparum* malaria. The parasitemia from examination of the slides were 0% on April 2, 0% on April 17, 0.06% on April 25, 5.5% on May 1 and 1.9% just before her death on May 2. Antibody titers against *P.f.* antigen were <1:4 on April 25, 1:256 on May 1 and May 2. These parasitological and serological results confirmed that she accidentally received transmission of *P.f.* through platelet transfusion.

The possibility of transmission of malaria through platelet transfusion has been discussed by Fajardo and Tallent (1974), who reported the existence of the parasite in a platelet of a *P.v.* infected patient by electron microscopy. Nevertheless, platelet packs may contain some red blood cells and parasites may be present in erythrocytes, platelets or both. The questionnaire which is used in obtaining the medical history of donors rarely includes inquiries on a past history of malaria. Special attention should be made for the safety of blood transfusion, taking into consideration of the danger of malaria transmission. This report is the first case of induced malaria resulting from platelet transfusion, and the 75th case of transfused malaria in Japan since 1935.

A—28

LIMITACION OF CLINICAL DIAGNOSIS OF MALARIA IN PHC PROGRAM

HIROSHI OHMAE[1], YURI J. UCHIDA[2], YUTAKA MIZUSHIMA[3], HIROMI KATO[3], TOSHIAKI TANAKA[4], ARBINO BOBOGARE[5], SAI KIRIMAOMA[5] AND AKIRA ISHII[6]
Bureau of International Cooperation, International Medical Center of Japan[1],
Department of Medical Zoology, Jichi Medical School[2],
Department of Internal Medicine, Toyama Medical and Pharmaceutical University[3],
Department of Internal Medicine, Nara Medical College[4],
Medical Reserach and Training Institute, Honiara, Solomon Islands[5]
and Department of Parasitology, National Institute of Health[6]

Malaria is one of the most sufferable infections in the world. About 550 million people are at risk from

malaria and over one million people are estimated to die from malaria every year. To prevent death from malaria, early clinical diagnosis and prompt treatment are recommended, and incorporated in the primary health care (PHC) systems. However, prompt treatments based on the results of early clinical diagnosis are not always enough to reduce the endemicity of malaria.

We investigated the accuracy of clinical diagnosis of malaria in 2 malaria endemic villages (A and B) in northeastern Guadalcanal in the Solomon Islands. In January 1993, the parasite rate (PR) was 73.1% and the sensitivity of clinical diagnosis was 36.7% in the A village. In the B village, the PR was 58.5% and sensitivity of clinical diagnosis was 37.5%. The residents in each village had been followed microscopically every 6 months. After the repeticious treatment based on the results of clinical diagnosis in the PHC activity, sensitivity of clinical diagnosis was reduced in 2 villages. After 12 months, the sensitivity of clinical diagnosis was reduced from 36.7% to 18.9% in the A village and was reduced from 37.5% to 15.4% in the B village. However, the PRs did not change and kept holoendemic levels.

Only by clinical diagnosis, many patients of malaria could not be diagnosed as malaria. Sensitivity of clinical diagnosis of malaria was reduced in PHC activity in holoendemic area. Many aymptomatic malaria parasite carriers, especially asymptomatic gametocute carriers could not be treated by the dignosis based on febrile clinical syptoms.

A—29

DNA DIAGNOSIS OF MALARIA IN THE SOCIALIST REPUBLIC OF VIETNAM

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

We have developed a colorimetric assay "microtiter plate-hybridization" (MPH) to detect amplified nucleic acids of malaria parasites on a microplate well. This method has high sensitivity and specifity more than microscopic examination. And this assay system allows us to detect and identify the four species of human malaria parasites. A pair of oligonucleotide primers were designed for the amplification of the conserved region of the gene coding for the 18S small subunit ribosomal RNA. Species-specific oligonucleotide probes were also designed and immobilized on microtiter wells. Target sequence of malaria parasites in human blood was amplified by the polymerase chain reaction (PCR) and the PCR-amplified product was captured by the species-specific probe on the microplate well. The biotin-streptavidin system was used to detect the captured materials. Positive samples gave yellow color by the chromogenic reaction. We have been carried out the epidemiological trial in the Socialist Republic of Vietnam, from July to August, 1994. Blood samples (10 μl each) were obtained from 154 donors by finger puncture. We have detected by two ways, microtiter plate-hybridization and microscopic examination using the blood samples. The microscopic examination was prepared two set of thin blood smear. One set was stained with acridine orange (AO) and the other set was stained with Giemsa. They were examined by the local microscopists. The 110 blood samples among the 154 samples were compared with MPH and AO, and 53 samples were compared with MPH and Giemsa. The difference between the results of MPH and AO is about 18%, and MPH and Giemsa is about 19%. Among 15 samples judged as *P.f./P.v.* mixed infection by MPH, only 4 samples was judged as *P.f./P.v.* mixed infection by microscopy. The case of mixed infection, judgement of species in microscopic examination was very difficult. Therefore, this MPH diagnostic method is indicated with effective tool on epidemiological research. On AO, we think that because of insufficient time to check smear, the mistakes happened.

It was well known that the malaria parasite was almost *P.f.* and *P.v.*, scarcely *P.m.*, in Vietnam. But we had found that 2 samples from within 29 samples in Phu Rieng was *P.m.* Therefore, we think that *P.m.* may

exist high rate depend on place.

We found a sample that was positive by microscopy, but negative by MPH. We think the sample might be a new malarial species which has different target region of 18SrRNA gene from other human malaria species.

A—30

ANALYSIS OF MITOCHONDRIAL DNA FROM *PLASMODIUM VIVAX*

KIYOSHI KITA[1], AKATSUKI KOKAZE[1], NATSUKO UE[1], SOMEI KOJIMA[1],
PATRICIA LIM[2] AND JOHN MAK[2]
Department of Parasitology, The Institute of Medical Science, The University of Tokyo[1]
and Biotechnology Centre, Institute for Medical Research, Malaysia[2]

Over the past decade, more evidence has accumulated to suggest an active role of the intraerythrocytic *Plasmodium* mitochondrion. In addition, recent effort to define the mitochondrial DNA of malaria parasites have revealed unique feature of the genome. It 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) and 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 mitochondrial DNA and its sequence analysis are difficult in the case of other human malaria, such as *P. vivax, P. ovale* and *P. malariae*. In this study, primers specific for *P. falciparum* COIII gene were used in polymerase chain reaction (PCR) to amplify a part of *P. vivax* COIII gene. Sequence data of the partial *P. vivax* COIII gene is then compared with those of *P. falciparum* and other organisms.

P. falciparum specific primers, which amplify 373 nucleotides coding the partial peptide of COIII (from Val-14 to Ser-138), were synthesized. Total DNAs of *P. vivax* were prepared from two patient, one Malaysian and one Malaysian student from India. PCR products with expected size were cloned into TA vector and sequenced. Alignment of the nucleotides of *P. vivax* COIII with published sequence of *P. falciparum* COIII showed nucleotide homology of 88%, while amino acid sequence comparison gave a homology of 91%. It is interesting to note that sequence data of COIII for both Malaysian and Indian isolates of *P. vivax* were identical, although relatively higher frequency of mutation in the mitochondrial DNA has been suggested. In contrast to the great similarity between two *Plasmodium* species, similarity between *P. vivax* COIII and human COIII (28%) was much lower than that between yeast COIII and human COIII (40%). Deletions were observed in the transmembrane helix II and two hydrophilic segments for both *P. vivax* and *P. falciparum*. These deletions are found only in the *Plasmodium* enzymes, 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.

A—31

USEFULNESS OF THE PCR METHOD WITH MICROTITER PLATE-HYBRIDIZATION IN THE CLINICAL PRACTICE OF IMPORTED MALARIA

MIKIO KIMURA[1], HIROFUMI MIYAKE[2] AND YUSUKE WATAYA[2]
Institute of Medical Science, University of Tokyo[1]
and Department of Pharmaceutical Sciences, Okayama University[2]

The novel and convenient PCR assay with microtiter plate-hybridization has been developed mainly by Wataya and Yamane. This method targets the 18S rRNA genes of malaria parasites that are specific for each species following amplification with the biotinylated primer set common to all four parasite species. Ten μl of blood was first hemolyzed with saponin and the DNA was dissolved with proteinase K treatment. Biotinylated PCR product is hybridized with specific probes that are coated onto microtiter plate wells and subsequently visualized after reaction with alkaline phosphatase-conjugated streptavidin and the substrate *p*-nitrophenyl phosphate. This PCR method was already applied to field studies in the Solomon Islands and Vietnam mainly by Wataya and others, and was found to be useful for the species-specific identification of malaria parasites especially in low parasitemia.

In this study this method was applied to imported malaria from various regions of the world. In some patients, serial blood samples were assayed and the results were compared with clinical findings and parasite densities. So far, all of 24 cases of falciparum malaria and 25 of 26 cases of vivax malaria were diagnosed species-specifically by this PCR assay. Also all of 7 cases of ovale malaria and 2 cases of malariae malaria were PCR-positive. By determining the blood samples taken in convalescent stages of some patients, the method was revealed to detect as few as 10 parasites/μl blood. In addition, there are 4 smear-negative, PCR-positive results in patients who had already taken anti-malarial medicine, suggesting these cases being in the convalescent stage of malaria. Thirty samples taken from non-malarial patients showed negative PCR results for each parasite species.

Thus, this method was shown to be extremely useful for clinical practice of malaria, e.g., diagnosis and species identification in low parasitemia, detection of mixed parasite infection, evaluation of anti-malarial treatment and also for the retrospective diagnosis of malaria after having taken anti-malarial medicine. These data also provide evidence that the method is a valuable tool for field studies.

A—32

THE PHAGOCYTOSIS OF *PLASMODIUM FALCIPARUM* AND TNF PRODUCTION BY HUMAN MACROPHAGES

TAKAYOSHI NAGAO, HARUKI UEMURA, TETSUO YANAGI AND HIROJI KANBARA
Department of Protozoology, Institute of Tropical Medicine, Nagasaki University

It's known well that *Plasmodium falciparum* (P.f.) induce TNF production of host's peripheral monocytes and tissue macrophages. Now it is regarded as the mechanism of TNF induction that the soluble substance released from malarial infected RBC at the period of rupture stimulate the monocytes in the circulation directly. But the phagocytosis of malarial parasites by reticuloendothelial macrophages in spleen and liver are large amount, so these stimulation are also speculated as important factor inducing TNF from tissue macrophages. So at this time we carried out the incubation of human peripheral monocytes with RBC infected with cultured P.f. and measured TNF induced into the supernatant of coculture and observed phagocytosis of parasites. And the infected RBC was separated into the soluble and insoluble fraction and TNF inducing ability of each fraction was compared. Next we derived tissue macrophages from human peripheral monocytes and measured the phagocytosis of parasites and the amount of TNF secretion and compared them with that of fresh isolated monocytes.

Methods: Cultured P.f. was synchronized to ring

form with 5% sorbitol and grew it to mature trophozoites and schizonts by going on the culture after synchronization. The monocytes isolated from human peripheral blood were stimulated with each infected RBC of different life stage obtained as above process, and we assayed TNF production and phagocytosis. Next the infected RBC was lysed by freeze-and-thaw method and soluble fraction was separated from insoluble fraction by high speed centrifugation and membrane filter. The human monocytes were stimulated with each fraction and TNF production was compared. And next human peripheral monocytes were incubated for 6 days then they differentiated into tissue macrophages. And similarly they were stimulated with infected RBC, and soluble, insoluble fraction from infected RBC and compared with fresh monocytes isolated from peripheral blood about TNF production and phagocytosis.

Results: When human peripheral blood monocytes were stimulated with infected RBC, the stage of late trophozoites and schizonts with new ring form caused monocytes to produce high value of TNF and high rate of phagocytosis of malarial pigments. From the result of experiment which monocytes were stimulated with soluble and insoluble fraction separated from infected RBC, it became clear that both fraction induced TNF and insoluble fraction, especially malarial pigments, were phagocytosed very easily by the monocytes. However, both soluble and insoluble fraction induced very little amount of TNF production from activated macrophages derived from peripheral monocytes, phagocytosis of insoluble fraction (malarial pigments) was accelerated conversely.

Conclusion: The factor which induce TNF production of human monocytes exists in the insoluble fraction besides soluble substance reported at past. And the acceleration of phagocytosis and decline of TNF production of derived macrophages suggested that phagocytosis of parasites is not a direct stimulation to induce TNF production.

A—33

HUMAN BLOOD LYMPHOCYTE RESPONSES TO *PLASMODIUM FALCIPARUM* INFECTION

*Rzepczyk, C.M. *Anderson K, *StamatiouS, *Townsend E, +Alloworth A,
+McCormack J, +Whitby M.
*Queensland Institute of Medical Research and *Dept of Medicine,
University of Queensland, Brisbane, Australia

Using flow cytometry, the response of human peripheral blood lymphocytes (PBL) to *Plasmodium falciparum* infection was studied in patients who presented at hospitals in Brisbane with acute *P. falciparum* infection. Some patients were followed longitudinally for up to six months after infection. We were particularly interested in the $\gamma\delta$ T cell response to malaria infection, although $\alpha\beta$ T cells, and natural killer (NK) cells were also chartered.

Peripheral blood lymphocyte profiles of malaria patients were variable. While $\gamma\delta$ T cells were elevated in some patients, they were not in others. These differences did not correlate readily with the patients' previous malaria history. The mean percentage of $\gamma\delta$ T cells in the blood of 17 patients during the acute stage of infection was 6.1 $\pm$ 4.7 SD (range 1-17), whereas the values obtained for 17 malaria naive control subjects was 3.8 $\pm$ 2.7 SD (range 1-9). In patients the largest fraction of $\gamma\delta$ T cells were $CD4^-$ and $CD8^-$, although there was a well defined population of $\gamma\delta$ T cells expressing CD8. The majority of $\gamma\delta$ T cells expressed CD45RO. This proportional representation of $\gamma\delta$ T cells subsets appeared comparable to that seen in malaria naive control subjects. During the acute stage of infection, the majority of $\gamma\delta$ T cells expressed HLA- DR antigens. This in marked contrast to the finding in control subjects where only a proportion of $\gamma\delta$ T cells showed HLA-DR expression. The high levels of HLA -DR expression on $\gamma\delta$ T cells were found to be maintained in some patients for at least two months after treatment. In some patients NK cells were elevated above the normal range with a maximum percentage of 35% being observed in one patient. There was also activation of $\alpha\beta$ T cells during infection. The relative contribution of the different sub-populations to malarial immunity and/or pathology still needs to be determined.

A—34

DRUG SENSITIVITY OF *PLASMODIUM VIVAX*

HIROYUKI AMANO[1], SAORI HIGASHI[2], TOSHIMASA NISHIYAMA[2],
SHIGEAKI ISHIZAKA[2] AND TSUNEJI ARAKI[2]
Department of Overseas Medical Services, Tenri Hospital[1]
and Department of Parasitology, Nara Medical University[2]

Drug-resistant strains of *Plasmodium falciparum* are well known around the world, although chloroquine remains drug of choice for *Plasmodium vivax* treatment even now. However, some problems have arisen recently, such as a chloroquine-resistant strain, and low sensitivity of sulfadoxine/pyrimethamine (SP) and primaquine in treating *P. vivax*.

This report presents our experience with the low sensitivity of *P. vivax* to SP and the drug sensitivity of *P. vivax* in literture is discussed.

A 21-year-old Japanese male student developed a fever (42°C) for 6 days after coming back from the Thailand-Myanmar border in April, 1993. Although treatment with SP in one hospital eliminated his malarial symptoms, fever recurred twice within 14 days after treatment and he visited our hospital 2 days after the second recrudescence. On admission, the patient had clear consciousness and no abnormal signs except hepatomegaly (2 cm). Important laboratory findings were; hemoglobin 11.0g/dl, platelet $28.3\times10^4/\mu l$, CRP 6.9mg/dl, FDP$<10\mu g$/ml, creatinine 0.9mg/dl, glucose 118mg/dl, total cholesterol 125mg/dl and LDH 654IU. Abdominal ultrasonicated echogrophy showed mild hepatosplenomegaly. Thin blood smear revealed trophozoites and gametocytes of *P. vivax* and parasitemia was 0.294%. Treatment with chloroquine (total 1,500mg base for 72 hours) was started on the second hospital day, followed radical therapy with primaquine (30mg× 14ds), and parasitemia completely disappeared. Clearance of fever and parasitemia required 36 hours and 72 hours, respectively. There were no side effects, and neither relapse nor recrudescence has occurred since then.

Our previous study showed that the cure rate of sulfamonomethoxine/pyrimethamine against *P. vivax* malaria is 100% and SP showed the same rate. Generally, although SP is effective, it is considered less effective than chloroquine against *P. vivax* and according to a Thai report, SP 2 tablet-therapy against *P. vivax* failed in 40% of cases. Additionally, this and a previous case of *P. vivax* malaria that we treated with SP 3tabs× 5days revealed that there seems to be a low SP-sensitive strain of *P. vivax*. Although our case was cured by chloroquine, cases of chloroquine-resistant *P. vivax* malaria have been reported in Papua New Guinea and Indonesia since 1989. Low primaquine-sensitive strains of *P. vivax* have been known in Southeast Asia and Latin America since the 1950's. Therefore, it is necessary to be more prudent in the treatment of *P. vivax* malaria.

A—35

AGE-SPECIFIC MANIFESTATION OF DRUG RESISTANCE OF *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN MALARIOUS ISLANDERS IN EASTERN MELANESIA

AKIRA KANEKO[1], GEORGE K. TALEO[2] AND HARUO KAMIYA[1]
Department of Parasitology, Hirosaki University School of Medicine[1]
and Malaria Section, Department of Health, Vanuatu[2]

Vanuatu is located at the southeast perimeter of the malarious band extending from Southeast Asia to eastern Melanesia. From 1988 to 1991 we conducted mass blood surveys in 33 areas of 11 islands in Vanuatu and examined a total of 11,590 islanders with overall parasite rate (PR) 13.5%: 679 *Plasmodium falciparum* (Pf), 847 *P. vivax* (Pv), 15 *P. malariae* (Pm), and 18 mixed infections. 165 of Pf cases had gametocytes. We analysed age-specific PRs. PR sharply increased from 0 to 1-2 years for both Pf and Pv. For Pv, PR was the

highest (15.9%) in subjects aged 3-4 years, and then dramatically decreased by age. For Pf, PR stayed in the range of 5.6-8.6% between age 1 and 19 years. As a result, a reversal of the Pf/Pv ratio was found at 10-11 years. Pf gametocytes were detected in all age groups but its PR was higher in subjects aged 1-14 years than in those more than 15 years.

For selected positive cases of the above surveys, we carried out drug resistance tests using the modified WHO methods. *In vitro* response of Pf to chloroquine was observed in 55 cases with 9 resistance (R) and 17 borderline cases. That to mefloquine was observed in 25 cases with no R and that to quinine in 36 with one R. *In vivo* response of Pf to chloroquine (600mg base on Day 0, 1 & 2 as adult dose: CHL) was observed in 153 cases with 2 R3, 5 R2 and 36 R1. The appearance rate of R2/R3 in subjects aged 0-4 years (15.2%) was significantly higher than that in more than 5 years (1.7%): $p<0.01$. The appearance rate of R1 in the former (51.9%) was also significantly higher than that in the latter (21.2%): $p<0.01$. *In vivo* response of Pf to Fansidar (pyrimethamine 75mg + sulfadoxine 1,500mg on Day 0 as adult dose: PYR/SDX) was observed in 172 cases with no R3, 5 R2 and 4 R1. During the follow-up to these Pf cases, Pv parasitaemia appeared in 24 cases after PYR/SDX administration, but in only 1 case after CHL. The appearance rate of Pv after PYR/SDX was the highest (33.3%) in subjects aged 0-4 years and 0.0% in those more than 15 years. *In vivo* response of Pv to CHL was observed in 102 cases with only one R2. That to PYR/SDX was observed in 58 cases with 2 R3, 6 R2 and 3 R1 without significant differences between age-groups.

For chemotherapy of residents in malaria-endemic areas, it is important to consider not only the drug resistance in the parasites but also the disease prevalence by age in the hosts. It seems that the formation of premunitinon by age suppresses the clinical manifestation of drug resistance.

A—36

IMMUNOSUPRESSION AND MALARIAL PARASITE

MIDORIKAWA YUTAKA[1] AND QUAZI MANJURUL HAQUE[2]
Suzuka University Medical Sciences & Technology[1]
and Department Public Health, School of Medicine, Mie University[2]

Relation between Deoxspergualin (DSG) an immunosupressive agent and malarial parasite was studied. Several chemicals incruding DSG, which are seemed to be the chandidate of malareal treatment, were tested both in vitro and in vivo. Inhibitive effect of DSG on malarial parasite in vitro was not good effect as in vivo, comparing other chemicals. So the mechanism of inhibition of DSG on malarial parasite was studied in vivo with using mice infected by *Plasmodium berghei*. Hematological results were collected in this study. The number of leucocyte and erythrocyte, amount of hemogulobin concentration and hematocrit % during infection and DSG treatment were counted and measured. The number of leucocyte was increased by malarial infection and decresed by DSG treatment. Same as the result of leucocyte number, erythrocyte, hemogulobin concentration and hematocrit % were decreased by DSG treatment. These fact shows that DSG inhibit cell segmentation of blood cells in mouse and especially, when immature erythrocyte production was inhibited by DSG, malarial parasite was also inhibited the infection because malarial parasite has the tendency of infecting the immature erythrocytes rather than the matured cells.

A—37

EPIDEMIOLOGICAL SURVEY OF CHAGAS DISEASE IN MACHARETY VILLEGE, BOQUERON PREF. PARAGUAY

AKIHIKO UCHIDA[1], J. MARTINEZ[2], YASUSHI KAWAKAMI[1] AND MASAO SANO[2]
Dept. Environmental Biology, Azabu Univ.[1] and IICS, Asuncion Univ.[2]

A total of 101 (2-78 years old) inhabitans of Macharety in Boqueron Prefecture, Paraguay, 47 men and 54 women, were examined immunologically and parasitologically along the field survey conducted. Results of immunological (ELISA) test revealed that sera from 15 men and 18 women showed positive results at a dilution of 1:80, while 10 of them replied that they were diagnosed as Chagas disease before. Although 11 persons (5 men and 6 women) replied that they had Romana sign, but only 4 of them showed positive results by ELISA test. A unilateral bipalpebral edema of the 7 persons was likely due to by other diseases.

Serological studies were performed among 101 people, from which 55 were subjected to ECG studies. Eight of them had been found to have ECG abnormalities, but only five of them were ELISA positive, the remaining three were ELISA negative. ECG abnormalities were found in just 9.0% and were rather lower when compared with other studies. The abnormalities found were as follows: Sinusal bradichardy with left-deviation (less than 55 beats per minute), Sinusal bradichardy, Left deviation, Complete blocking of right branch with forward-left hemiblocking.

As for triatomine in houses, 84 (93.3 %) of 90 people replayed "yes" and 72 (88.0 %) said that they had been stuck by the bugs in bed during the night. In every house surveyed, 2-26 (on average 11.6) bugs were found and indentified as *Triatoma infestans* the major vector of *T. cruzi*.

A—38

SERODIAGNOSIS OF CONGENITAL CHAGAS' DISEASE IN ACUTE PHASE

SACHIO MIURA[1], TUNEARI SEKIGUCHI[1], TOSHIAKI KAMIYA[2],
KAORU MATAYOSI[3] AND TUTOMU TAKEUCHI[1]
Department of Tropical Medicine and Parasitology, School of Medicine, Keio University[1],
Department of infectious disease, Christian University Bolivia[2]
and Hospital Colonia Okinawa N° I, Internal Medicine[3]

Chagas' disease, coursed by the parasitic protozoan *Trypanosoma cruzi* (*T. cruzi*), is a sever problem for public health, that's affects several million of people in Central and South America. The prevalence of seropositivity for Chagas' disease has declined in parallel with vector control activities in this area. Generally transmission route are three: (1) Vectorial, (2) Transfusion, (3) Vertical transmission. Today's great problem is congenital infection of *T. cruzi* coursed by vertical transmission in productive age group. Recent report of the congenital Chagas' disease has been 2~5% in urban and 23~81% in endemic area in Bolivia. Congenital Chagas' disease of newborn have been poor aspects for diagnosis and need more studies for standardization of serodiagnosis in acute phase of them.

25 sera of newborn were collected from umblical cord at birth and trypanosoma was detected by Strout method and the mothers corresponding with them were showed positive for IgG by ELISA. In this time we studied for newborn cases by immunofluorescence antibody tests (IFA), ELISA and Immunoblotting. Then 24 out of 25 congenitally newborn had detected IgG antibodies to *T. cruzi* but for IgM positive were showed in only 6 cases. Also, immunoblotting exhibited 13 positive and 12 negative out of 25 cases. Only one case showed all results of serodiagnosis were negative although parasitemia had been positive. Comparing these results with chronic Chagas' disease studied in Recife (Brazil) showed different pattern, that is immunological examinations of chronic Chagas' disease in Recife had good

agreement with various serodiagnosis.

In conclusion from these studies, we suggest that IFA is more stable methods than others. A method for early detection of infected newborns is essential for treatment.

Further studies are contemplating the problem in these aspects.

A—39

T-LYMPHOPROLIFERATIVE RESPONSE OF THE PATIENTS WITH CHAGAS' DISEASE TO EPIMASTIGOTE ANTIGEN OF *T. CRUZI* IN GUATEMALA

KENJI HIRAYAMA[1], EDMUNDO VELASQUEZ[2], MARTIN GIL[2], TAMARA OBISPO[3], JULIO ARGUETA[4], DIANA FREIRE[5], MARGARITA PAZ[2], TETSUO YANAGI[6], KAZUKI OGATA[4], EITARO HORI[1] AND ISAO TADA[7]

Department of Medical Zoology, Saitama Medical School[1], San Carlos University[2], Roosevelt hospital[3], JICA-Guatemala research project of tropical diseases[4], Institute of atomic energy[5], Nagasaki University Institute of Tropical Medicine[6] and Kyushu University Faculty of Medicine[7]

Proliferative response of peripheral mononuclear cells of the patients with Chagas' disease against epimastigote antigen of *T. cruzi* was investigated using 10 outpatients of Roosevelt hospital with heart disease from endemic area in Guatemala and using 20 individuals who had been diagnosed as trypanosomiasis by serology in Santa Maria Ixhuatan where JICA project had been going on since 1990.

There observed a good correlation between serology (Indirect Hemagglutination Test; IHA, Seroimmunodiagnostic, Co, USA) and T lymphoproliferative response to epimastigote antigen except the fact that even seronegative individuals sometimes responded to the antigen moderately. Those sero negative responders were significantly responded compared with Japanese non-exposed population. For further identification of the major antigens of epimastigote of *T. cruzi* by T cell level and as well as B cell level, we prepared partially purified fractions (32 fractions) after passing through ion-exchange chromatography (DEAE-memsep, Milipore, USA) by using sodium chloride gradient (0-0.4N). Two seropositive individuals showed different pattern of responsiveness to each fractions except fraction 29 that stimulated both. The interesting finding was that when we examine their antibody reactivity to those fractions, both did not show any reactivity against fraction 29. Thus it was suggested that fraction 29 preferentially stimulated T cells rather than B cells in those patients. Further study should be needed using more patients with different clinical forms of Chagas' disease.

A—40

STUDIES ON THE DETECTION OF DENGUE VIRAL GENOME BY RT-PCR

KEN-ICHIRO YAMADA, YUKI ESHITA[1], MITSUHIRO NUMATA, SADAO YABE AND TADAHIKO KITANO

National Institute of Health, [1]Kurume University, School of Medicine

The present study was carried out to detect D1, D2, D3 and D4 viral genome from the cultured fluid of infected mosquito cells, patients' sera and the preparation of mosquito extract by rapid RT-PCR method. RT-PCR was performed as previously described (Morita, et al., J. Clin. Microbiol. 29:2107-2110, 1991). C6/36 cell line was grown at 28 C in MEM supplemented with 10 % fetal bovine serum and 0.2 mM of non-essential amino

acids and TRA-284-SF cell line was grown in a serum -free medium that consisted of an equal volumes of Leibovitz (L-15) and tryptose phosphate broth. All four serotypes of dengue viral genomes could be detected from the cultured fluid of infected two mosquito cell lines. It was detected that the cell-associated viral genome was 24 hr and the extra-cellular viral genome was 30 hr after infection in TRA-284-SF cells, respectively. D1 viral genome was detected from the sera of patients 3 days after the onset of disease. Although the amplified DNA band was detected from the virus solutions, the amplified DNA product could not be detected from the preparation which were prepared the mixture of an equal volume of virus solution and uninfected mosquito extract. The inhibitor effect could be removed by heating (100 C, 5 min.) or treatment of 30 ug/ml trypsin solution. Obvious DNA band was observed on the preparations which were treated with heating (100 C, 5 min.) in case of the infected mosquito samples. These results suggest that the inhibitors containing mosquito extract were some heat-labile proteins. All four serotypes of dengue viruses could be plaque assayed with TRA-284-SF cell cultures using the overlay media of MEM contained 1 % methylcellulose.

A—41

SEQUENCES OF E/NS1 GENE JUNCTION FROM FOUR DENGUE-2 VIRUSES OF NORTHEASTERN THAILAND AND THEIR EVOLUTIONARY RELATIONSHIPS WITH OTHER DENGUE-2 VIRUSES

KYAW ZIN THANT, KOUICHI MORITA AND AKIRA IGARASHI
Department of Virology, Institute of Tropical Medicine, Nagasaki University

We determined the 240 nucleotide sequences of the E/NS1 gene junction of four dengue-2 uiruses by the primer extension dideoxy chain termination method. These viruses were isolated from the dengue patients with different clinical severities in Nakhon Phanom, Northeastern Thailand in 1993. The results were compared with the 52 published dengue-2 sequences of the same gene region. Sequence divergence of four new isolates varied from 4.17% to 5.42% compared with dengue-2 prototype New Guinea C strain whereas it varied from 5.42% to 6.67%, and 6.67% to 7.09% when compared with Jamaica 1409 strain and PR159/S1 strain, respectively. Most of the nucleotide substitutions were found at the 3rd position of the codons which only caused silent mutations. All 56 isolates studied were classified into five genotypic groups by constructing the dendrogram. The results indicated that four new isolates from Northeastern Thailand belong to genotype II of dengue virus serotype 2, and were most closely related to prototype New Guinea C strain.

We also observed the variation in nucleotide and amino acid sequences among clusters of isolates (Thailand-1980, Malaysia-1989 and Thailand-1993) which were obtained from the dengue patients with different clinical severities. Significance of these genetic differences have been discussed to draw the possible correlation between genetic variability and virulence factor.

A—42

CLASSIFICATION OF *LEISHMANIA* PARASITES, USING KDNA FINGER PRINTING METHOD BY ARBITRARILY PRIMER POLYMERASE CHAIN REACTION

TATSUYUKI MIMORI[1], JIICHIROU SASAKI[1], MOTOMI NAKATA[2] AND HIDEYUKI SAYA[1]
Department of Oncology, Kumamoto University School of Medicine, 2-2-1 Honjo, Kumamoto 860, Japan[1]
and Biomedical laboratory, Sumitomo Electric Industries[2]

Leishmaniases are widely distributed in Central and South America and Europe, Africa and Asia except South-East Asia, where they present a considerable public health problem. The identification of mor-

phologically similar parasites responsible for different clinical features of the disease is very important. Identification techniques, isoenzyme electrophoresis, monoclonal antibodies, DNA hybridization, molecular karyotypes, restriction fragment length polymorphism, polymerase chain reaction have been performed in diagnosis and epidemiological survey of leishmaniasis. These techniques, however, have some defects like as a necessity of a large quality of materials, complicated procedure and/or difficulty for judgment. Recently, PCR finger printing method by arbitraily primed polymerase chain raection (AP-PCR) was developed on the analysis human DNA polymorphisms. Moreover, some preliminary studied of classification by this method were reported on bacteria and parasite. In this study, we have performed the identification of *Leishmania* parasites in South America using AP-PCR.

We used in this study WHO reference and standerd *Leishmaia* strains and *Leishmania* isolates from wild mammals and humans in Ecuador, South America were also examined for utility value of this method. These isolates have been checked previously, using isoenzyme electrophoresis and monoclonal antibodies. *Leishmania* promastigotes were cultured in Schneider's medium, harvested parasites were resuspended in SE buffer (0.15 M NaCl, 0.1 M EDTA, pH 8.0) lysed with sarkosyl and digested with proteinase k at 60 °C. The kinetoplast DNA (kDNA) net-works were collected by centrifugation (16,000 rpm for 90 min at 4 °C), extracted by phenol chloroform and precipitated by ethanol. These kDNA were sued for templates of AP-PCR. The quontity (10 -500 ng) of templates were checked using two *Leishmania* kDNAs. The six primers were tested. Amplification was carried out in a thermal cycler (Perkin-Elmer) for initial 5-10 cycles of low stringency (1 min at 94 °C, 1 min at 37 or 42 °C, 1 min at 72 °C) and 25-30 cycles of high stringency (1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C). At the end of the PCR cycles all tubes were incubated for 7 min at 72 °C to allow the amplification process to go to completion. The PCR product was analyzed by 6 % polyacrylamide gel electrophoresis and silver staining.

The kDNAs fragment bands were shown more clear on polyacrylamide gels under the following AP-PCR amplification condition; 10 cycles of low stringency (1 min at 94 °C, 1 min at 37 °C, 1 min 72 °C) and 30 cycles of high stringency (1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C). All experiments were performed on this amplyfy condition. Six primers were tested using different specices, and primer- LS3 was more useful one for identification between *Leishmania* complex. From these results, the AP-PCR may be valuable approach for identifying and distinguishing *Leishmania* parasites.

B—1

A SURVEY OF HOOKWORM AND *STRONGYLOIDES* INFECTION FOR ADULT FARMERS LIVING SURROUNDING SURABAYA CITY, AND FOR ADULT FACTORY WORKERS IN SURABAYA CITY, INDONESIA

KAORI KOGA-KITA[1,2], DANIEL HUSEA[3], SRI SUBEKTI[3], IG.N. GDE RANUH[3], SHIRO KASUYA[2] AND YUZO TAKAHASHI[2]
Department of Public Health, Faculty of Medicine, Kagoshima University[1], Department of Parasitology, Gifu University School of Medicine[2] and Tropical Disease Research Center(TDRC), Airlangga University[3]

Recently, the agar plate method has been shown to be successful in detecting *Strongyloides stercoralis* in fecal materials, while traditional methods have been reported to be unreliable. We have been reporting that the agar plate method is very efficient, based on results of our previous studies in Thailand and Cambodia, not only for its reliability but also for its field applicability in developing countries. We have also been reporting that hookworm infection is also detected successfully by the agar plate method in the prevalent areas. We have been studying about improvement of procedure as an appropriate technology for developing countries, so that the usage of the agar plate method would be globally advantageous in the diagnosis of strongyloidiasis and hookworm infections that are broadly prevalent in tropical area in the world. We have been reporting that since prevalence rates of *Strongyloides* in our previous study in Thailand and Cambodia were much higher than

previous surveys performed without the agar plate method, it is suggested that global prevalence of *Strongyloides* infection is much higher than the present estimation.

In this study, we made a survey of hookworm and *Strongyloides* infections for adult farmers living surrounding Surabaya City, and for adult factory workers in Surabaya City, Indonesia, using the agar plate method.

Hookworm infections were detected in 108(31%) of 344 fecal samples from adult (over 20 years old) villagers living surrounding (within 40 km) the city, and in 17(4%) of 417 samples from adult factory workers. *Strongyloides* infections were detected in 69(20%), and in 9(2%), respectively. From these results, it was shown that *Strongyloides* and hookworm infection rates were quite high among farmers living in the villages in this area. On the other hand, these infection rates were low among residents of the city, and there was a significant difference between farmers and factory workers.

Samples from diarrhea cases of small children (under 5 years old) were also examined. However no hookworm or *Strongyloides* infections were detected in 75 samples from a hospital. These parasites did not seem to be playing a significant role in agents of the diarrhea in small children in this area.

Prevalence rates of *Strongyloides* were again much higher than previous surveys performed without the agar plate method also in this area, among adult farmers. These results supported the suggestion that global prevalence of *Strongyloides* infection is much higher than the present estimation.

B—2

SERO-EPIDEMIOLOGICAL SURVEY OF HUMAN TOXOCARIASIS IN NEPAL, BANGLADESH AND THAILAND

K. KONDO[1], N. AKAO[1], T. OHYAMA[1], T. OKAZAWA[2], Y. TSUDA[3],
K. YANO[4], M.R. AMIN[5] AND W. CHOOCHOTE[6]
Dept. of Parasitology, School of Medicine, Kanazawa University[1],
Dept. of Public Health, School of Medicine, Kanazawa University[2],
Faculty of Pharmaceutical Sciences, Kanazawa University[3],
Inoue Hospital[4], Dept. of Micro-biology, Faculty of Medicine, Dhaka University[5]
and Dept. of Parasitology, Faculty of Medicine, Chaing Mai University[6]

The authors have been engaged in sero-epidemiological survey of human toxocariasis in tropical zone, especially, Southest Asia. We have got 344 sera in Nepal (98 sera in Dharan and 246 sera in Nepalganj), 37 sera in Dhaka, Bangladesh and 80 sera in Chaing Mai, Thailand. We conducted the serological test by ELISA against ES antigen of *Toxocara canis* larvae (TcnLES).

The rate of the antibody titer against TcnLES antigen is Nepalganj, Nepal (8.94%) and the lowest in Chaing Mai, Thailand (2.50%). The mean antibody against TcnLES is the highest in Dhaka, Bangladesh (1.49±0.34) among other for investigated areas including those in Ishikawa, Japan (control).

These for areas have hot and humid climate and are epidemic in soil transmitted parasitic diseases. Although there is no clear on the antibody titer against TcnLES between control and four areas, it is obvious that the antibody titer of these four areas are relatively higher than in control area.

B—3

AN IMMUNOLOGICAL STUDY OF CYSTICERCOSIS IN GUATEMALA

HIDETAKA ICHIKAWA[1], NOBUAKI AKAO[2], LETICIA CASTILLO[3], EDMUNDO VELASQUES[4], YOUICHI ITO[5], KAZUKI OGATA[3], ISAO TADA[6] AND KAORU KONDO[7]
Department of Medical Zoology, Faculty of Medicine, Kanazawa Medical University[1],
Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University[2],
JICA project[3], San Carlos University[4], Department of Parasitology, Faculty of Medicine, Kitazato University[5],
Department of Parasitology, Faculty of Medicine, Kyushu University[6]
and Department of Parasitology, Faculty of Medicine, Kanazawa University[7]

We examined the antigenicity and its nature of *Cysticercus cellulosae,* a major health problem in Guatemala, which causes serious lesions in central nervous system and skeletal muscles. Antigenic compositions of the cyst fluid (F), scolex (S), membrane (M), and whole cyst (C) of the cysticercus from pig obtained in "E" area of Guatemala were compared by using SDS -PAGE with those from F obtained in China aiming at the selection of F-antigen suitable for serodiagnosis. All these four antigens were composed of more than twenty bands and no differences were seen in the protein components among them in spite of minor differences in protein concentrations. The main components of Guatemalan F were of 60 and/or 54 kDa, while those of the Chinese were 100, 60 and 48 kDa judging from the quantiative aspect of protein concentration. ELISA assay was performed by using F as the antigen on 78 randomly selecsed sera from the inhabitants of Santa María Ixhuatán. Seven (8.97%) in the samples showed positive and the average antibody titer was 1.18 ± 0.14. Among 43 sera from suspected cysticercosis cases, stored at $-80°C$ in the Division of Malaria, Ministry of Health, ten (23.26%) samples revealed positive and the titer was 1.26 ± 0.29. There was no significant difference between two groups in their average titers. An immunoblot assay of serum sample out of 4 from this village with higher titers recognized 48, 42, 39 and 23 kDa bands in F.

B—4

DIFFERENTIAL DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN ECUADOR

SHIGEO NONAKA[1], ATSUSHI HOSOKAWA[1], ABDUL MANAN BHUTTO[1] AND YOSHIHISA HASHIGUCHI[2]
Department of Dermatology, Research Center of Comprehensive Medicine, Faculty of Medicine,
University of the Ryukyus[1] and Department of Parasitology, Kochi Medical School[2]

Cutaneous leishmaniasis is a protozoal disease infested by sand flies and widespread in tropical zone of the world. Clinically, there are three types, that is, visceral, mucocutaneous and cutaneous type of leishmaniasis. We have investigated the skin changes of cutaneous leishmaniasis in Ecuador since 1988. Most popular cutaneous change was ulcer, but non-ulcered cutaneous changes such as papules, plaques and nodules were also seen frequently. The symptom we have to be most careful for differential diagnosis of this disease was an ulcer formation. Ulcus cruris, a leg ulcer, is one of the diseases which we should be careful for differentiation. The patients with the leg ulcer often work for agricultural purposes in the farm of endemic areas. The clearly demarcated ulcers are formed on the tibial region. Seven patients had been diagnosed as cutaneous leishmaniasis and treated previously with meglumine antimonate. However, the ulcer of these patients still continued at the time of first examination. Parasitological examination did not show any positive reaction for leishmaniasis. The most important point of differential diagnosis in the leg ulcer was the presence of ithcing sensation in the surrounding area of the ulcers. There was high incidence of leg ulcer in old age group. Generally, no indurations exist around the ulcer, whereas clearly detectable induration were in the patients with leg ulcer treated with topical injection of meglumine antimonate. In younger age group, impetigo and

traumatic injury were also considered as a cause of ulcer, but no induration existed around these ulcers. In endemic areas, we saw one patient with chromomycosis, and a patient with basal cell carcinoma. The patient with basal cell carcinoma had been diagnosed as mucocutaneous leishmaniasis because of the invasive lesion of the nasal mucosa. The differential diagnosis of cutaneous leishmaniasis should be performed carefully not only by clincal examination, but also by parasitological and histopathologycal examinations.

B—5

FIRST CASE OF DIFFUSE CUTANEOUS LEISHMANIASIS IN ECUADOR

ABDUL MANAN BHUTTO[1], SHIGEO NONAKA[1] AND YOSHIHISA HASHIGUCHI[2]
Department of Dermatology, Faculty of Medicine, University of the Ryukyus, Okinawa[1]
and Department of Parasitology, Kochi Medical School[2]

Diffuse Cutaneous Leishmaniasis (DCL) is a specific type of leishmaniasis in which the lesions disseminates all over the body. We report a case of DCL having the nodules and plaques on all over the body since 5 years. Recently, he developed the verruca vulgaris lesions on the hand and foot. The parasites were isolated and characterized as the *Leishmania (Leishmania) mexicana*. Skin test with leishmania antigen was negative, but tuberculin, candidine and trichophytone antigens showed positive reactions. Histologically, macrophages were heavily parasitized and vacuolated, and infiltrated in all over the dermis. By immunohistochemical stain, T cells were located in the sections. Electron microscopically, HPV particles were found in the verruca lesions. Morphology of the parasites were similar with that of normal leishmania parasites, and they were phagocytized by the macrophages and few parasites were seen under degeneration. Association of virus infection and negative delayed reaction with leishmania antigen represents the immunological depression of the patient. The unresponsiveness of T cells to the leishmania antigen may be due to the host defect(s) which are unknown. The high load of parasites in the sections may be due to the T cell defect which ultimately results in the spread of disease.

B—6

GENETIC REGULATION OF CUTANEOUS LEISHMANIASIS IN MICE INFECTED WITH *LEISHMANIA AMAZONENSIS*

KEN KATAKURA, NAOHIRO WATANABE AND HIROSHI OHTOMO
Department of Tropical Medicine, Jikei University School of Medicine

Outcome and subsequent development of the skin lesion of cutaneous leishmaniasis is varied with *Leishmania* species and genetic background of the host. Little information, however, is available in terms of genetic regulation of American cutaneous leishmaniasis. Using mouse models, we analyzed genetic regulation of cutaneous leishmaniasis caused by the infection with *Leishmania amazonensis*. Promastigotes of *L. amazonensis* were inoculated into the shaven rumps of mice, and host's susceptibility was determined with several inbred mouse strains by measurement of the skin lesion diameters. BALB/c and C57BL/6 mice exhibited a susceptible phenotype with progressive larger lesions throughout the 120 day infection period, whereas SJL/J mice showed really a resistant phenotype with no apparent lesions. The F1 (BALB/c × SJL/J) mice exhibited an intermediate lesion growth phenotype. In the F1 × BALB/c backcross progeny, 11 and 8 mice showed susceptible and intermediate phenotypes, respectively. In the F1 × SJL/J progeny, 34 mice exhibited an intermediate phenotype and 38 animals showed a resistant phenotype. Furthermore, linkage analysis was carried out with respect to the *Lsh* (*Bcg*) and *Scl2*, which are mapped on chromo-

some 1 as a natural resistant gene for *L. donovani* infection and on chromosome 4 as a control gene for *L. mexicana* infection, respectively. For this purpose, we used microsatellite repeats [(CA)n] as chromosome markers, which are demonstrated to be polymorphic among different mouse strains and can be detected by polymerase chain reactions. Fifty to seventy-two backcross mice (F1 × SJL/J) were analyzed with microsatellite markers in the vicinity of *Lsh* and *Scl2* loci. As a result, mouse susceptibility to *L. amazonensis* infection was linked to neither the *Lsh* nor *Scl2*. These results suggest that murine cutaneous leishmaniasis caused by *L amazonensis* infection is regulated by a single gene different from genes reported for regulating leishmaniasis by other *Leishmania* species.

B—7

FAMILY EXAMINATION OF LEPROSY PATIENT IN LOW ENDEMIC AREA FOR THE DISEASE IN ECUADOR

ATSUSHI HOSOKAWA[1], SHIGEO NONAKA[1], JUAN J. ALAVA P[2], EDUARDO A. GOMEZ L.[3], HUGO M. JURADO S.[4] AND YOHIHISA HASHIGUCHI[5]

Department of Dermatology, Research Center of Comprehensive Medicine, Faculty of Medicine, University of the Ryukyus, Ninhihara, Okinawa 903-01, Japan[1], Universidat Tecnica de Portoviejo, Portviejo, Manabi, Ecuador[2], Departamento de Medicina Tropical, Facultad de Medicina, Universidad Catolica Santiago de Guayaquil, Guayaquil, Ecuador[3], Centro Nacidal de Medicina Tropical, Guayaquil, Ecuador[4] and Department of Parasitology, Kochi Medical School, Nankoku, Kochi 783, Japan

Four cases of leprosy patients were seen in an aerea endemic for cutaneous leishmaniasis, Los Ranchos, the Department of Manabi. Two cases of them (borderline lepromatous leprosy and indeterminate leprosy) in a single family were reported. Other two leprosy patients were examined in the current examination. Futher more, the existence of other four leprosy patients in the village was determined. From the result, it was suggested that family examination of leprosy patient might be usefull for detection of leprosy in a low endemic areas for the disease, such as Department of Manabi. A nine banded armadillo that were kept by the family had no acid-fast bacilli in the liver.

Case 1: a 41-year-old man. Present histoly: In 1977, erythema appeared on the abdomen and a doctor diagnosed him as borderline lepromatous leprosy in 1982. Present condition; Infiltrated erythemata on the face, pea sized reddish nodules on the earlobe, erythemata on the trunk were observed. Deformity of nose, hands, feet and deep ulcer on the soles were observed. The anesthesia and dry skin were observed on almost all over the body excep for the axillary, the epigastric, the lower abdomen, the inguinal, the perineal and the anal region. The ichthyosis-like change was seen on the waist, back, the extensor aspect of the legs and the feet. Hair loss was not observed at any lesion of the body surface. Clinical examination; The Lepromin test was negative. Value of anti phenolic glycolipid (PGL)-I and antilipoarabinomannan (LAM-B) antibodies were all positive. Histological findings; Rete peg disappeared. Clear subepidermal clear zone was observed. Though relatively large number of epithelioid cells and lymohocytes infiltrated in the dermis, epitheliod cell granuloma was not observed. Bacterial index showed 3+; SFG index 5 (1-2-1). Nasal scraping was negative.

Case 2: The patient was a 12-year-old female. A daughter of case 1. Present history. When we examined the family of case 1 patient, hypopigmented fleckle with anesthesis on the extensor aspect of the left thigh was noticed. Hair loss was not observed. The type of leprosy was indeterminate group. Clinical examination; Lepromin test was undeterminable. Value of PGL-I and LAM-B antibodies were positive except anti-PGL-IgM. Histological findings; small number of lymphocytes infiltrate around the capillaries and appendages. There was no epithelioid cell granuloma in the dermis. No acis bacilli was observed by acid fast staining.

B—8

HEALTH EDUCATION IN THE CONTROL OF SCHISTOSOMIASIS HAEMATOBIUM IN COAST PROVINCE, KENYA: EFFECT ON KNOWLEDGE, ATTITUDES AND PRACTICES

KAZUHIKO MOJI[1], YOSHIKI AOKI[2], TOSHIKI AWAZAWA[2], MASAAKI SHIMADA[3], NGETHE MUHOHO[4] AND MELANIE KATSIVO[4]
Department of Public Health, School of Medicine, Nagasaki University[1],
Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[2],
Department of Medical Zoology, School of Medicine, University of Environmental and Occupational Health[3]
and Kenya Medical Research Institute[4]

The health education is expected to complement the control measures of schistosomiasis. It helps people understand that their own behaviour is a key factor in spreading the disease and the changes in their practices reduce prevalence of disease. The present study deals with the effect of the health education on knowledge, attitudes and practices of people in an endemic focus of schistosomiasis haematobium in Kenya. In 1991, before the start of health education programme, 190 male or female heads of the households, 96% of the total households of the study area, were interviewed. Among them 138 heads were interviewed again in 1993, after the completion of education for 1 year. The educational materials used in our health education activities were the manual of health education in schistosomiasis published by WHO and our own version of video tepes.

Health education improved people's perception and knowledge of schistosomiasis. In 1991, 62% of villagers recognized schistosomiasis as the extreamly serious disease. In 1993, the perception of schistosomiasis as a serious disease increased to 85%. Only 58% of people reported accurately the cause of the disease in 1991. In 1993, 85% of villagers acquired the knowledge that the disease is caused by the infection of schistosome. There was a remarkable decrease in the number of the villagers who did not know the sign and symptom of the disease (from 36% to 6%).

The changes in attitudes and practices were also significant. The number of villagers who used the safe water for bathing increased from 88 to 96%. The amount of tap water used in a household increased from 9.9 to 20.9 litter/day/adult. Number of villagers with latrin in their houses increased from 11 to 24%. However, both the number of villagers who took the urine examination and the percentage of the villagers who took the drug did not increase. The prevalence of disease in our study area remained relatively high, although the mass-chemotherapy has been repeated every 2 years.

The present study revealed that health education cultivated the knowledge of schistosomiasis in the villagers, but did not modify their attitudes and practices so as to reduce the risk of infection in the study area.

B—9

STUDIES ON SCHISTOSOMIASIS JAPONICA IN ORIENTAL MINDORO, PHILIPPINES (1) SEROEPIDEMIOLOGICAL SURVEY IN SCHOOL CHILDREN WITH BLOOD TAKEN ON FILTER PAPER

HAJIME MATSUDA[1], YUICHI CHIGUSA[1], HAJIME YOKOI[1], MASASHI KIRINOKI[1],
KAZUO YASURAOKA[2], LYDIA E. BUGAWAN[3] AND BAYANI L. BLAS[3]
Department of Medical Zoology, Dokkyo University School of Medicine[1],
Institute of Basic Medical Sciences, University of Tsukuba[2]
and Schistosomiasis Control Service, Department of Health, Philippines[3]

An enzyme-linked immunosorbent assay (ELISA) with a crude antigen from lyophilized *Schistosoma japonicum* eggs was used in a seroepidemiological survey for schistosomiasis japonica in 9 elementary schools in the town of Victoria and Pola, Oriental Mindoro, Philippines in August 1994. A small amount of blood was taken from each school child of the 1st to 6th grades through finger prick and was allowed to dry on quantitative sampling filter paper (Toyo Rosi Type I). Before testing this dried blood specimen was dissolved overnight in PBS Tween containing 1% BSA at 4°C to get 1: 200 dilutions. A negative serum pool and known positive control sera were included in each test plate. An optical density of more than 0.3 was considered to be positive. A total of 1,308 children from the different localities (barrios) of Victoria and Pola were examined, and 398 or 30.4% of them were found positive for antibodies to *S. japonicum*. Positive rates in the eight elementary schools of Malabo, San Narciso, Urdaneta, Duongan, Central II, Bethel, Malayas and Pakyas in Victoria were 75.4%, 47.1%, 40.7%, 33.7%, 24.1%, 22.7%, 13.6% and 10. 7%, respectively. The prevalence at Tagbakin in Pola was 36.0%. The positive rate varied greatly with schools and was higher in boys than in girls. The results of this survey indicated that the barrios of Malabo, San Narciso, Urdaneta and Duongan located near the Lake Naujan were the areas of the highest risk. This is probably due to high vector transmission potential in these barrios since *O. quadrasi* prefer a marshy area for their breeding sites.

In the present study, significantly more positives were detected with ELISA than with stool examinations, thus suggesting that repeated stool examination should be done for ELISA positives.

B—10

INTERMEDIATE SNAIL CONTROL IN *SCHISTOSOMA HAEMATOBIUM* ENDEMIC AREA OF KENYA

KENICHI TAKAHASHI[1], SHINICHI NODA[2], SHIGEHISA HABE[3],
NGETHE D. MUHOHO[4] AND YOSHIKI AOKI[5]
Hokkaido Institute of Public Health[1], Department of Medical Zoology, Faculty of Medicine, Kagoshima University[2],
Department of Parasitology, Faculty of Medicine, Fukuoka University[3],
Kenya Medical Research Institute[4]
and Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[5]

Attempts were made to control snail population (*Bulinus globosus*) in Mwachinga and Mtsangatamu Villages in Kwale District, Kenya.

In Mwachinga Village, the snail control was carried out in upper part of the Kadingo River. The Kadingo River dries up in the hot dry season, with subsequent formation of small pools. Towards the end of the dry season, these pools may dry up completely. However, snail hosts can survive under the vegetation which maintain an appropriate temperature and humidity. Snails in the pools were eliminated by scooping, and river bed was dried by clearing vegetation during the

period between October 1992 and October 1993. Snail surveys were carried out twice each month at 7 sites. Annual number (June-May) of collected snail before snail control was 666-3,855. Only 5 snail were collected during the period between June 1993 and May 1994.

In Mtsangatamu village, the modification of river stream was carried out on January and February 1994 in the part of the Mtsangatamu River of which flow is perennial. The snail habitats were reduced by clearing of water plants and concentrating the stream water in a narrow channel. Snail survey were carried out every three months at 25 sites. The annual number of collected snails in 1992 and 1993 was 507 and 430, respectively. No snail hosts were collected by snail survey after modification.

B—11

ASSAY OF SERUM TYPE IV COLLAGEN IN *SCHISTOSOMA JAPONICUM* INFECTION

HIROSHI OHMAE[1], YASUSHI MATSUZAKI[2], TAKESHI NARA[3],
YUJI IRIE[4], BAYANI L. BLAS[5], GERUNDIO P. PORTILLO[6],
ORLAND S. SY[6] AND KAZUO YASURAOKA[4]
Bureau of International Cooperation, International Medical Center of Japan[1],
Institute of Clinical Medicine, Tsukuba University[2],
Institute of Medical Sciences, Tokyo University[3],
Institute of Basic Medicine[4],
Schistosomiasis Control and Research Service, Manila[5]
and Schistosomiasis Hospital, Leyte, The Philippines[6]

We previously reported ultrasonographic (US) and serologic abnormalities in patients with *Schistosoma japonicum* in 1990. The US liver images were classified into 4 patterns, according to the development of periportal fibrosis. Among various serum indicators of liver damage, the serum levels of total bile acid (TBA) correlated with the development of liver fibrosis in *S. japonicum* infection.

Type IV collagen is a main component of basement membrance. In the liver, type IV collagen is present in the basement membrance in the parenchyma area. It has been reported that the serum type IV collagen levels reflect the liver fibrosis and the validity of IFN treatment in the patients with HCV.

US examinations concomitant with biochemical serum analysis including analysis of type IV collagen were performed on 52 patients at Schistosomiasis Hospital in Leyte, Philippines in 1990 and 1994. A significant increase in the levels of serum type IV collagen was detected in the patients diagnosed as periportal fibrosis by US. High levels of type IV collagen were also found in some of the patients dignosed as no or mild periportal fibrosis by US. The serum type IV collagen level was more sensitive to detect the periportal fibrosis than the serum levels of TBA and Procollagen-III -Peptide. A significant decrease in the serum type IV collagen level was also found after praziquantel treatment. The serum type IV collagen level was proved to be one of the most sensitive tool to monitor the severity of hepatic fibrosis caused by *S. japonicum* infection, as well as the improvement resulting from praziquantel treatment.

B—12

GENETIC STUDY OF JAPANESE ENCEPHALITIS VIRUSES FROM MALAYSIA

HIDEAKI TSUCHIE[1], KAZUMASA ODA[2], INDRA VYTHILINGAM[3], RAVINDRAN THAYAN[3], BALASUBRAMANIAM VIJAYAMALAR[3], MANGALAM SINNIAH[3], MOHAMMAD MOFAZZAL HOSSAIN[1], TAKASHI KURIMURA[1] AND AKIRA IGARASHI[4]
Research Institute for Microbial Diseases, Osaka University[1],
Japan International Cooperation Agency[2],
Institute for Medical Research[3],
Malaysia and Institute of Tropical Medicine, Nagasaki University[4]

Ten Japanese encephalitis (JE) virus strains were isolated from *Culex* mosquitoes caught in Selangor, Malaysia in 1992. In order to analyze the 10 JE virus strains isolated from Malaysia in 1992, we examined a 240 nucleotide sequence from the pre-M gene region of the strains, and studied their genetic relationships to the other JE virus isolates from a variety of geographic area in Asia. No difference in the nucleotide sequences was observed among our 10 JE virus isolates from Malaysia. Our JE virus strains belong to the largest genotypic group that includes strains isolated in temperate regions such as Japan, China, and Taiwan. WTP/70/22 strain isolated from Malaysia in 1970 and JE-827 strain isolated from Sarawak in 1968, belong to another distinct genotypic group. Our Malaysian JE virus strains, the WTP/70/22 strain, and the JE-827 strain might have evolved from a common ancestor and have circulated in mosquitoes in Malaysia. Alternatively, new genotype of JE virus including our Malaysian isolates might have been extending in Malaysia recently via introduction of the virus by migrating birds or by international transportation systems such as jet airplane.

B—13

A 27 AMINO ACID CODING REGION ON THE C-TERMINAL OF JAPANESE ENCEPHALITIS VIRUS E-PROTEIN EXPRESSED IN RECOMBINANT *ESCHERICHIA COLI* POSSESSES NEUTRALIZING EPITOPE(S)

SAID ALI SEIF, KOUICHI MORITA, SACHIKO MATSUO,
FUTOSHI HASEBE AND AKIRA IGARASHI
Department of Virology, Institute of Tropical Medicine, Nagasaki University,
1-12-4, Nagasaki, 852, Japan

In order to localize denaturation-resistant neutralizing epitope(s) in the C-terminal 180 amino acids of Japanese encephalitis virus (JEV) E-protein, four recombinant clones encoding different or overlapping nucleotide sequences were constructed by PCR from a recombinant plasmid pS22. The amplified fragments were cloned into PCR 1000 vector, and then transferred into Escherichia coli expression vector pRIT2T. The inserted genes were expressed as fusion proteins with protein-A and examined for their antigenicity and immunogenicity by western blotting and mouse immunization respectively. Among the four recombinant fusion proteins, the highest neutralization antibody titre was obtained by the one expressed by the recombinant clone pRIT2T-B3, which carried the coding sequence of amino acid number 373-399 of JE virus E protein. The results indicate that this short region of 27 amino acids sequence near the C-terminal of JEV E protein possesses neutralizing epitope(s) and is hereby earmarked as a promising candidate for a synthetic peptide vaccine.

B—14

ISOFORMS OF mRNA FOR LARGE SUBUNIT OF CYTOCHROME B558 DETECTED IN cDNA LIBRARY OF HUMAN GRANULOCYTES

SHOICHI SUZUKI, ATSUSHI KUMATORI AND MICHIO NAKAMURA
The Department of Biochemistry, Institute of Tropical Medicine Nagasaki

Reactive oxygens play important roles in defence against invading bacteria and in the regulations of various biological responses in hosts. Most of these oxygens are generated by NADPH oxidase on the surface of phagocytes upon stimulation. Membrane-bound cytochrome b558 is an essential constituent of NADPH oxidase for the generation of superoxide anion. It is a heterodimer of a 91-kD glycoprotein (large subunit) and a 22-kD polypeptide (small subunit). As the large subunit is expressed only in myelogeneous cells at the late stage of differentiation, it should have a key role in many responses associated with reactive oxygen species. We therefore tried to isolate isoforms of mRNA for the large subunit that may have different types of information for regulatory domains.

For getting homologous clones to mRNA of the large subunit, cDNA library of human granulocytes was screened by plaque hybridization under low stringency. Seven clones were obtained and sequenced on a Pharmacia automatic sequencer.

Clone S1, S13 and S18 had identical sequences from exon 6 to exon 12, exon 7 to exon 13 and exon 12 to exon 13, respectively of the cDNA for the large subunit. Clone S7, S15 and S16 contained identical and non-identical sequences to large subunit cDNA. The clone S7 had first adenine of TATA box in putative promoter region. This result therefore indicates an alternative initiation site for mRNA for the large subunit. Clone S3 has mosaic sequences, 5' portion of which seems to encoding a novel peptide containing more than 132 amino acids, and 3' portion of which encodes 40 amino acids identical to the C-terminal of the large subunit. This clone suggests the presence of an isoform of mRNA for the large subunit.

B—15

ANALYSIS OF NOVEL CHRONIC GRANULOMATOUS DISEASE

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

Patients with chronic granulomatous disease (CGD) suffer from severe bacterial and fungal infections beginning in early childhood. Their phagocytes cannot kill microbes efficiently due to deficiency of superoxide-generating NADPH oxidase activity. The NADPH oxidase is a multi-component enzyme consisting of a large (gp91-*phox*) and a small subunit (p22-*phox*) of cytochrome b558, p47 and p67, and genetic defects in any one of the components lead to CGD. Defects in gp91-*phox* are most common in CGD because its gene lies on X- chromosome. We found a novel X-linked CGD patient of 61 year-old man who was not diagnosed until the patient had first severe infections at the age of 43 years. To clarify the cause of the CGD, we analyzed genetic defects of NADPH oxidase in the patient's leukocytes using biochemical and molecular biological technique.

Method

Superoxide generative activity in peripheral neutrophils was measured by chemilumi-nescence after stimulating them with phorbol myristate acetate (PMA). Expression of each component for NADPH oxidase was analyzed by western blot analysis of neutrophil extracts using specific antibodies raised against them. Amount of gp91-*phox* and p22-*phox* mRNA was determined by Northern blot analysis using total RNA extracted from peripheral mononuclear leukocytes. Protein coding region in gp91-*phox* mRNA was amplified into three overlapped fragments by RT-PCR method. The amplified DNA fragments were cloned into pBluescript and

sequenced by the dideoxynucleotide chain termination method.

Result and Discussion

Patient's neutrophils did not generated superoxide in response to PMA. The patient showed undetectable amount of cytochrome b558 subunits on a western blot analysis of neutrophil extracts. The patient's neutrophils express cytosolic components of NADPH oxidase (p47 and p67) normally. On a Northern blot the patient showed apparently low levels of gp91-*phox* mRNA in mononuclear leukocytes, but the amount of p22-*phox* mRNA was normal. We did not found any abnormalities in coding region of gp91-*phox* mRNA of the patient, nevertheless, on other cases decreases in its mRNA accompanying with abnormality in the sequence. These results indicate that major cause of the CGD is due to decrease in transcriptional rate of the gp91-*phox* gene derived from genetic defects in regulatory regions governing transcription of the gene.

B—16

EFFECTS OF DIETHYLCARBAMAZINE AGAINST *WUCHERERIA BANCROFTI* IN FIJI: FIVE ROUNDS OF ANNUAL SINGLE-DOSE TREATMENT VS. A COURSE OF 28-DOSE TREATMENT SPREAD OVER 2 YEARS

J.U. MATAIKA[1] AND E. KIMURA[2]
Wellcome Virus Laboratory, Suva, Fiji[1] and Aichi Medical University[2]

Parasitological and clinical effects of annual single-dose treatments with diethylcarbamazine citrate (DEC) given at 6 mg/kg in 5 consecutive years (a total dose of 30 mg/kg) and a 28-dose DEC treatment at 5 mg/kg spread over 2 years (a total of 140 mg/kg) were compared using about 15,000 people in 82 villages in Fiji, where diurnally subperiodic *Wuchereria bancrofti* transmitted mainly by *Aedes polynesiensis* and *Aedes pseudoscutellaris* is endemic. Assesed at 5 years after the commencement of treatment, the former scheme reduced microfilaria (mf) rate from 6.5% to 0.9% (an 86% reduction) and mf density (geometric mean of mf count/60 μl of blood among the mf positives) from 21 to 5 (a 76% reduction). The 28-dose scheme reduced mf rate from 11.6% to 0.9% (a 92% reduction) and mf density from 29 to 5 (an 83% reduction). In the annual treatments, the reduction in the mf rate and density was gradual but steadily progressive; in the 28-dose treatment, the reduction was quick and remarkable, but a clear increase in the mf rate and density was observed 2 years after the completion of the treatment. It is possible a few additional annual doses would surpass the efficacy of the multi-dose scheme. The 5 annual treatments were effective in reducing filarial fever, though the 28-dose treatment showed better clinical effects. In control villages, where no treatment was given for 3 years, both mf rate and density increased. After serving for 3 years as a control group, the people were treated with 2 rounds of annual single-dose treatment with DEC. The study indicates that 5 rounds of annual single-dose treatment, with the total dosage of only about a fifth of the extensive 28-dose treatment, resulted in comparable effects to the latter.

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B—17

FUNDAMENTAL SURVEYS FOR THE UTILIZATION OF MEDICINAL PLANTS ACAINST FILARIASIS IN KENYA (MINIREVIEW)

MAKI, J.[1], ITO, Y.[1], KOFI-TSEKPO, W.M.[2], AKAI, K.[3], FUJIMAKI, N.[4], MITUI, Y.[4] AND AOKI, Y.[4]
Dept. Parasitology, Kitasato University School of Medicine[1],
Kenya Medical Research Institute[2],
JICA Project team leader[3]
and Dept. Parasitology, Kitasato University School of Medicine[4]

Obstinate parasitic diseases like filariasis have annoyed Kenyan inhabitants, especially those in rural areas. If drugs with high efficacy and low prices without severe side effects were readily available, the present situation would be improved. It appears that plant-derived drugs would meet this requirement. From this viewpoint, the present authors have started preliminary surveys and preparation for the possible utilization of medicinal plants against filariasis in Kenya. This communication is a brief review of the survey of an endemic area of the infection with *Wuchereria bancrofti*, information on herbal therapy of filariases, and medicinal plants harvested.

The researchers of Kenya Medical Institute (KEMRI) and Japan International Cooperation Agency (JICA) visited the southeast region of Kenya for the epidemiological survey of *W. bancrofti* infection and the collection of medicinal plants. In Kwale district there have been found three villages Lutsangai, Gandini and Dzivani where the inhabitants are suffering from the infectious disease caused by *W. bancroftic* (Gachihi et al., 1994, Jpn. J. Trop. Med. Hyg. 22:41). Mosquitoes sprayed down in houses and collected were dissected under a stereoscopic microscope and observed under a light microscope for the possible detection of the 3rd -stage larvae. The vectors were demonstrated to be three kinds of mosquitoes, one species of *Culex* and two species of *Anopheles* (Mwandawiro, personal communication).

Published papers on medicinal plants with possible efficacy against filariases from the viewpoints of ethnobotanical and experimental studies have been collected by the present authors (Maki & Kofi-Tsekpo, 1994, JICA Progress Report). A number of plant species, for instance *Andrographis paniculata* (Dutta & Sukul, 1982, J. Helminth., 56:81-84) and *Zingiber officinale* (Dutta & Sukul, 1987, J. Helminth., 61:268-270) have been examined in the efficacy of the extract against filariae in India where filariases are rampant. The bark of Indian medicinal plant, *Azadirachta indica* is said to be useful for the treatment of filariasis there (Comley, 1990, Trop. Med. Parasit. 41:1-9).

In East Africa, three medicinal plants, *Cyphostemma nierense, Psychotria tanganyikensis* and *Xeroderris stuhlmannii* are thought to be useful for the treatment of elephantiasis (Kokwaro, 1993, Medicinal Plants of East Africa, 2nd edition, 401, Kenya Literature Bureau). In kenya, the leaves of *A. indica* have gained popularity among inhabitants hoping treatment and prophylaxis of malaria. The possible efficacy of the leaf extract against filariae cannot be denied to the best of the present author's knowledge.

For the start of the new work, Kofi-Tsekpo & Maki (1994, Research Proposal submitted to KEMRI, 3 -4) selected about 20 species of plants to be tested in the efficacy against filariae. Medicinal plants, *A. indica, Clausena anisata, Edithcolia* sp., *Maytenus* sp., *Tabernaemontana stapfiana* and *Toddalia asiatica* collected in Taita Hills and Taveta District were brought back to the laboratory in KEMRI for the preparation of crude drugs (Maki & Kofi-Tsekpo, 1994, JICA Progress Report).

The extracts will be subjected to in vitro and in vivo assays. Jirds infected with *Brugia pahangi* are readily used for in vivo tests. In vitro maintenance of adult filariae has been studied by a number of workers (see the review by Maki (1991, Kitasato Arch. Exp. Med., 64:179 -182)). The NI medium or a 1:1 mixture of NCTC135 and IMDM is thought to be one of the best media for the in vitro maintenance of adult *B. pahangi* under the gas phase of 5% CO_2 in nitrogen or air. The worms will be maintained in vitro so that the effect of the extracts on their movement and microfilarial production might be closely observed.

B—18

A NEW METHODS FOR DETERMINATION OF DIETHYLCARBAMAZINE AND IVERMECTIN IN THE BLOOD BY ENZYME-LINKED IMMUNOSORBENT ASSAY

YOSHINORI MITSUI[1], YASUNORI FUJIMAKI[1] AND YOSHIKI AOKI[1]
Department of Parasitology, Institute of Tropical Medicine, Nagasaki University[1]

A new simple and sensitive methods for determination of diethylcarbamazine (DEC) and ivermectin (IVM) were successfully developed. The methods are on the competitive enzyme-linked immunosrobent assay (ELISA). DEC and IVM were structurally modified and conjugated to bovine serum albumin for use as immunogen. An antiserum specific for DEC and IVM were elicited in mice and rabbits respectively.

For determination of DEC in the fluid, DEC-poly-L-Lysine conjugate was expressly prepared for use as the solid phase antigen. A competitive indirect ELISA was conducted by simultaneously incubating DEC with anti-DEC antibody over DEC-ploy-L-Lysine solid phase and then determining the bound mice immunoglobulin with sheep anti-mouse immunoglobulinG peroxidase conjugate. Response for DEC in the resulting competitive curve was between 1 and 1000ng/ml.

For determination of IVM in the fluid, IVM-biotin conjugate was expressly prepared. A competitive ELISA was by simultaneously incubating IVM and IVM-biotin with anti-IVM antibody over goat anti rabbit IgG and then bound IVM-biotin detected by Avidin peroxidase. Response range for IVM in this system was 0.1-10ng/ml.

The sensitivity of our methods are as high as those of gas chromatography and HPLC.

An immeasurable advantage of our methods is that the methods require only 10μl of serum for accurate determination of drug concentration in the blood.

B—19

ARE THERE SPORADICALLY ENDEMIC FOCI OF LYMPHATIC FILARIASIS IN THE CARIBBEAN BASIN OF CENTRAL AMERICA?

SERGIO HURTARTE[1], ISAO TADA[2], J. ONOFRE OCHOA A.[1], SALVADOR HERRERA[3], SHINJIRO HAMAMO[2], RYU MATSUO[4], NOBORU MASUDA[4] AND KEITOKU BABA[4]
Malaria Division, Ministry of Health, Guatemala[1],
Department of Parasitology, Faculty of Medicine, Kyushu University[2],
National Hospital, Puerto Barrios[3]
and Kyushu University Association of Tropical Medicine[4]

Lymphatic filariasis have been found distributing widely in Caribbean basin such as Puerto Rico and Dominica. The disease was considered to have been transported from Africa by the slave trade. In Central America, however, lymphatic filariasis were ever reported only from Costa Rica. Butts (1947) investigated Puerto Limon area of this country in 1946 and found 1-15% of the residents were positive for microfilariae of *Wuchereria bancrofti* in Cieneguita, Jamaica, Bataan and Quepos. No other findings on lymphatic filariasis in Central American countries except Costa Rica. In 1993, some of us observed two cases of leg elephantiasis in the urban area of Puerto Barrios, a Caribbean port of Guatemala. Due to this observation record, a preliminary survey was done at Puerto Barrios in July, 1994. An interview study at various institutions and streets revealed two cases of typical leg elephantiasis, one resident of Puerto Barrios (53 year old male) from Livingston and the other (36 y.o. female) living in Mariscos near Izabal lake. A night blood survey on randomly selected 56 inpatients from the vicinity of Puerto Barrios and admitted to National Hospital revealed negative. In the blood examination, approximately 30 cmm of blood from the fingertip was microscopically examined. The present study could not find positive microfilaremic persons, while the presence of

leg elephantiasis from eastern coastal regions of the country will suggest sporadic distribution of lymphatic filariasis in Caribbean side and Lake Izabal basin in Guatemala, Central America.

B—20

RATES OF MICROFILARIA PREVALENCE AND CLINICAL FILARIASIS IN CHUUK (FORMERLY TRUK) STATE, FEDERATED STATES OF MICRONESIA

MAMORU FUJIWARA[1], KIOSI ANIOL[2] AND EISAKU KIMURA[1]
Department of Parasitology, Aichi Medical University[1]
and Department of Health Services, Chuuk State, Federated States of Micronesia[2]

In order to determine the present rate of filariasis, we carried out blood and clinical surveys on *Wuchereria bancrofti* infection. Since the report by Pipkin (1953), no systematic state-wide survey has been made for 40 years in Chuuk. A total of 2193 people (about 5.1% of the total State population) in 14 villages on 9 islands was examined for microfilaria (mf). The average mf rate of those examined was 2.6%. The villages on the islands located near the capital, Moen, showed low mf rates of less than 1%. On the other hand, the villages located relatively far from the capital, showed moderate to high mf rates. High mf rates of 7-10% were obtained in 3 villages on 3 islands. The mf rate of males (3.2%) was significantly higher than that of females (1.7%) ($p=0.03$). The highest mf rates were observed among males of age ≧20 years (6.2-10.3%). We studied 466 adult males of age ≧15 years for chronic clinical signs. The average rates of hydrocele, elephantiasis and endema were 3.4%, 0.4% and 0.9% respectively. There was a positive correlation between the rates of clinical filariasis and mf rates of the villages ($r=0.622$, $p<0.02$).

In the 1930s, filariasis was not an important disease in this area and Esaki (1938) even reported that there was no autochthonous case of filariasis in Micronesia. However, in 1951-52, mf rates in Truk District was 12-27% (Pipkin, 1953). This remarkable increase of mf rate might be explained partly by a large-scale immigration of Japanese, many of whom were from Okinawa where *W. bancrofti* was highly endemic. After Pipkin's study, Ando *et al.* reported the prevalence rate of 7.9% in 1982. Thus, our mf prevalence rate (2.6%) indicates that the mf rate has been on the decrease at least in moen and neighboring islands. Urbanization and improvement of life conditions might be the main reason.

B—21

STATISTICAL OBSERVATIONS ON CHYLURIA PATIENTS FOR THE RECENT TWENTY YEARS IN DEPARTMENT OF UROLOGY, KAGOSHIMA UNIVERSITY; INPATIENTS AND OUTPATIENTS BETWEEN 1974 AND 1993

YOSHIHIRO MIZUMA, ITARU MIYAWAKI AND YOSHITADA OHI
Department of Urology, Faculty of Medicine, Kagoshima University

In this report, we present statistical observations of chyluria patients for the recent twenty years between 1974 and 1993 in Department of Urology, Kagoshima University.

Comparative data of 1974 and 1993 shows tendency of aging and decrease in number of chyluria patients as follows: average age, 55.3 years old and 68.6 years old, number of patients, 34 and 11, percentage of chyluria patients for all out patients, 2.0% and 0.5%.

In our department, Nagata and Okamoto et al. investigated the lymphatic flow of chyluria patients using lymphography for determination of the etiology of the disease in 1967. They revealed prominently dilated, kinked and complicated lymphatic vessels with back flow of contrast media into the renal calyces. Organic obstruction of thoracic duct was not recognized. It has

been suggested that these pathological status of lymphatic channels result in stasis of lymph flow, with the subsequent development of lymphatic pressure. This may product fistularisation of lymphatic vessels into the urinary tract.

It was declared that filarial infestation was eradicated by 1975 in Kagoshima prefecture. But chyluria is still one of the important urological diseases here in the southern part of Kyushu Island, because chyluria is a chronic manifestation of filariasis.

B—22

CLINICOPATHOLOGICAL CHARACTERISTICS OF FOUR CASES OF PULMONARY DIROFILARIASIS

HIROSHI HIRANO[1], TERUMASA SASHIKATA[1] and TAKEO MATSUMURA[2]
Department of Pathology, Hyogo Medical Center for Adults[1]
and Department of Medical Zoology, Kobe University School of Medicine[2]

Pulmonary dirofilariasis, which is caused by *Dirofilaria immitis*, the dog heartworm, is a rare disease. However, it is being diagnosed more frequently each year, and the differential diagnosis of this disease and malignancies is of increasing importance. To the best of our knowledge, only about 60 cases have been reported in Japan. Four cases of pulmonary dirofilariasis were diagnosed at the Hyogo Medical Center for Adults in the period 1984 to 1993. Chest x-ray films taken during health check-ups showed a single spherical nodule 10 to 30 mm in diameter in each patient. All lesions were in the peripheral portion of the inferior lobe of the right lung. All patients lacked clinical symptoms and reported no contact with dogs. In three cases, results of hematological examinations were within normal limits, and in the remaining case, mild eosinophilia was found. Biochemical tests of blood gave normal results. Tests with an antibody to the causative agent were not done. Cytological and bronchoscopic examinations of the lungs gave no abnormal findings. Therefore, the dirofilariasis could not be readily differentiated from malignant disease without surgical resection of the lesion. Pulmonary dirofilariasis is not life-threatening, so resection should be avoided, when possible. Nevertheless, all four patients underwent surgery (lobectomy in three cases, and resection of the lesion in one case). The pathological evaluation of the resected specimens made possible a definitive diagnosis. The elastica van Gieson stain, Masson trichrome stain, silver stain, and hematoxylin-eosin stain were used. The silver stain was the most useful for showing recognizable worm structure: a smooth cuticle, well-developed musculature, an internal coelom, and intestinal and genital tracts. The patients seemed to have been infected several years earlier, because some larvae had degenerated and were not recognizable except for their smooth cuticle and musculature. We undertook immunohistological investigations of the operative specimens, which did not react with alpha-smooth muscle actin, but did react with the serum of a rabbit hyperimmunized with *D. immitis*. According to the literature, cross-sections of many dirofilariasis lesions show necrosis, endoarteritis, eosinophilia, and fibrosis. Each of our patients had one or more of these findings. The parasites were associated with thrombus formation within the pulmonary artery. Coagulation necrosis of this artery surrounded the worms. Pulmonary infarctions are usually wedge-shaped on chest x-ray films because the arteries embolized by a thrombus supply a wedge-shaped area. Risher et al. have suggested that pulmonary dirofilariasis shows coin-shaped lesions on x-ray films because the worms release a toxin that causes arteritis followed by infarction.

We thank Dr. E. Konishi for the hyperimmunized rabbit serum and Dr. S. Uga for his preparation of *D. immitis* microfilariae embedded in BJ-4.

B—23

BASIC STUDIES ON THE MONGOLIAN GERBIL AS A SUSCEPTIBLE HOST TO FILARIAL INFETION (13) SENSITIVITY TO DIABETOGENIC SUBSTANCES

KEIJIRO SHIROTA[1], KATSUYA HAGA[2], KOICHIRO FUJITA[2],
MASUMI SHIMIZU[2,3] AND KAZUHIRO SHICHINOHE[2,3]
Tobu Co-medical Technical College[1],
Department of Medical zoology, Faculty of Medicine, Tokyo Medical and Dental University[2]
and Department of Laboratory Animal Science, Nippon Medical School[3]

We reported that encephalomyocarditis virus M variant, which induced diabetes in mice, did not cause hyperglycemia in the Mongolian gerbil (*Meriones unguiculatus*) although it arose some lesion in the pancreas of the animal. We examined sensitivity of the gerbil to diabetogenic substances using alloxan and streptozotocin in the present study.

Fifty to 200 mg/kg of alloxan or streptozotocin were intravenously injected into three to 4 month-old gerbils. Bodyweight, food and water intake and blood glucose level were measured during 1 month and histological observation were performed.

More than 100 mg/kg of both chemicals induced hyperglycemia, polyposia and polyphagia in the gerbils since 3 days after injections. Degrees of hyperglycemia were dose-dependent. The animals injected with more than 150 mg/kg of alloxan or 200 mg/kg of streptozotocin became weakened and some of them died. Those data suggested that the optimum dose of alloxan and streptozotocin were 125 and 150 mg/kg, respectively to induce experimental diabetes in gerbils. Since pyknosis and degranulation of B cells of pancreas were observed in histological examination, lack of insulin induced by lysis of B cells might caused crisis of diabetic symptoms in gerbils.

B—24

BASIC STUDIES ON THE MONGOLIAN GERBIL AS A SUSCEPTIBLE HOST TO FILARIAL INFETION (14) THE ORGAN WEIGHTS OF THE WILD -COLORED GERBIL AND THE COAT COLOR MUTANTS

MASUMI SHIMIZU[1,3], KAZUHIRO SHICHINOHE[1,3], KAZUYOSHI KATO[2],
KATSUYA HAGA[3] AND KOICHIRO FUJITA[3]
Department of Laboratory Animal Science[1] and Department of Emergency and Critical Care Medicine, Chibahokuso Hospital[2],
Nippon Medical School
Department of Medical zoology, Faculty of Medicine, Tokyo Medical and Dental University[3]

We performed an anatomical study of Monogolian gerbils including the coat color mutants to establish baseline data of this animal and to know whether their anatomical feature relate to their high and wide susceptibility to parasites.

Fifty each of male and female gerbils having agouti, albino, black or other kind of coat color were used in this study. Those animals were 3 to 12 months old and they had been used for controls of other experiment or reproduction. The organ weights of the heart, lung, liver, spleen, pancreas, kidneys, adrenals and thymus were measured after each animal was bled under ether -anesthetization. Each organ/body weight ratios was also calculated.

Those data of the coat color mutants were comparable to those of the wild-colored gerbils. But the thymus of Mongolian gerbils was remarkably different from that of mice or rats. The weight of this organ generally reduced by age in mice or rats, but weight loss of the thymus was very little in the Mongolian gerbil and mature one still had a certain size of the thymus. It seemed to be a peculiar character of this animal and it may related to its susceptibility to various parasites.

We also immunohistologically examined a surface

antigen of the thymocytes of the gerbil including the coat color mutants using anti-Thy-1.1 and anti-Thy-1.2 monoclonal antibodies. Thy-1 antigen has been known as a cell marker for T-lymphocyte in mice and it consists of 2 types in mice, i.e. Thy-1.1 and Thy-1.2. Each strain of mice has either Thy-1.1 or Thy-1.2. Cortical thymocytes of the gerbil were strongly positive to Thy-1.1, whereas the response to Thy-1.2 was completely negative. The coat color mutants of the gerbil showed the same response. The results indicated that the Thy-1.1 antigen existed in the Mongolian gerbil and distribution of Thy-1 antigen did not have the coat color variations in the gerbil.

B—25

A NEW APPROACH FOR ANALYSIS OF HUMAN SWEATING

MUNENORI SHIMAZU[1], TAKAAKI MATSUMOTO[1], MITSUO KOSAKA[1],
NOBU OHWATARI[1], KATSUHIKO TSUCHIYA[1], YOSHITO UEYAMA[2,3,4],
KOJI URANO[2,3], YUKO KATAKAI[2,3] AND MUNEO SAITO[3]
Department of Environmental Physiology, Institute of Tropical Medicine, Nagasaki University[1],
Kanagawa Academy of Science and Technology[2], Central Institute for Experimental Animals[3]
and Department of Patholgy, School of Medicine, Tokai University[4]

Inducing of eccrine sweating of human skin transplanted into several strains of immuno-deficiency mouses' dorsal skin was investigated by intra-cutaneous administration of chemical stimulants. Experiments were carried out in an environmental chamber (Ta:26°C, rh:60%) without anesthesia. Using Minor's method, sweat spots were visualized and recorded with a video macroscope (NIHON KHODEN:VMS-1300). For inducing sweat, 0.01ml of different kinds of chemical stimulants (adrenaline, pilocarpine, atropine and pilocarpine) were administered intra-cutaneously. One chemical was used in a day to induce sweating. In a typical case, sweat volume induced by each chemical was in the following order; pilocarpine > atropine+pilocarpine > adrenaline > no chemical. VIP was also tried, however, none of response of sweating was induced. Histological investigation of the transplanted human skin was also performed in HE stain. In the histological specimen, eccrine sweat glands were found in rather good shape. Blood vessel and a bit of degenerate marks of nerve fibers existed around eccrine glands. It was confirmed that eccrine sweat glands in the transplanted human skin were considerably well kept its original structures and maintained the moderate function of sweat secretion. From this finding, it was supposed that eccrine sweating of transplanted human skin was dominantly cholinergic and slightly adrenergic under the condition of the lacking of innervation.

B—26

ANALYSIS OF LONG-TERM HEAT-ACCLIMATIZATION IN TROPICAL INHABITANTS -COMPARISON OF SWEAT RESPONSE AMONG JAPANESE, THAI, AND AFRICANS-

TAKAAKI MATSUMOTO[1], MASAKI YAMAUCHI[2], MITSUO KOSAKA[2],
DAVID SAM[2], NOBU OHWATARI[2], ZONG-WEI LUO[2],
YU CAO[2], AKIHIRO TAIMURA[2], EIKO KANEDA[2]
AND JEONG-BEOM LEE[2]
Department of Thermal Adaptation[1] and Department of Environmental
Physiology[2], Institute of Tropical Medicine, Nagasaki University

In order to clarify the mechanisms of long-term acclimatization to hot environment, sweat response and heat tolerance in tropical inhabitants were examined. Ten Thai, 6 Africans (3 Tanzanian, 1 Kenyan, 1 Ghanian and

1 Senegalese) and 11 Japanese were the subjects in this study. In a chamber at 26.6°C and 33% rh, sweat was induced by leg immersion into a hot bath at 43°C for 30 min. Oral and skin temperatures were measured with thermistors and local sweat rates on the chest and abdomen were measured with capacitance hygrometer -ventilated capsule method. Two of 6 Africans did not sweat during 30 min heat load, therefore, heat load was applied for another 10 min. Sweat onset time in Africans (21.60±8.22 min, mean±SD) and in Thai (16. 60±5.95 min) were significantly longer than that in Japanese (9.04±2.27 min). Local sweat volume in Africans as well as in Thai was significantly smaller than that in Japanese. Local sweat volume on the chest and abdomen were 10.16±5.00 mg/cm² and 6.81±4.16 mg/cm² in Japanese, 1.39±0.97 mg/cm² and 2.37±1.29 mg/cm² in Thai and 2.71±1.63 mg/cm² and 1.68±0.88 mg/cm² in Africans, respectively. There was no significant difference in the initial oral temperature before heat load among Japanese, Thai and Africans. Oral temperature in Africans as well as in Thai was kept lower compared with that of Japanese during heat load. There was no significant difference in threshold oral temperature for sweat onset among Japanese, Thai and Africans. From these results, it is concluded that though the amount of sweat is smaller in tropical inhabitants, heat loss ability is better compared to that in Japanese.

B—27

VASODILATION OF THE TAIL SKIN INDUCED BY ISOPROTERENOL IN CHRONIC SPINAL RATS

KATSUHIKO TSUCHIYA AND MITSUO KOSAKA
Department of Environmental Physiology, Institute of Tropical Medicine,
Nagasaki University

Effect of isoproterenol (adrenaline β-agonist) on the tail artery was studied in cervical spinal rats. The cervical cords of male Wistar rats were transected at the level of C8/Th1 under pentobarbital anesthesia. Spinal rats were reared in the room of 30°C. After the spinalization, body weight decreased progressively. Heart rate (HR) was calculated by the R-R interval of ECG. Rectal (Tre), tail skin (Ttail) and air (Ta) temperatures were detected by thermistor probes under the unrestrained condition. Isoproterenol hydrochloride (Isop, Proternol-L, Nikken Kagaku, Tokyo) 0.1mg/kg was injected intraperitoneally to rats after 2-3 weeks of the spinalization. Under resting condition, mean HR (M±SE, N=5) was 290±17 beats/min. By the injection, HR increased by about 50% and high level of HR was sustained for 30 minutes (min) and declined toward the initial level. Before the injection of Isop to conscious rats, mean Tre was 37.8±0.4°C. By the injection of Isop, Tre increased gradually. Mean Tre was 38.4±0.9°C after 60 min of the injection. The index of blood flow in the tail, dTtail (dTtail=Ttail-Ta) was 3.7±0.6°C before the injection. By the injection of Isop, dTtail initially decreased and thereafter gradually increased to be over the value before the injection. Mean dTtail was 1.9±0. 8°C after 10 nin, and 4.7±0.7°C after 60 min of the injection. These changes in dTtail were statistically significant ($p<0.05$) compared to the value before the injection. In this study, the injection of Isop induced both vasoconstriction and vasodilation in the tail of the chronic spinal rats. Fregly (1983) suggested that the tail vasodilation by Isop was not direct effect on vasculature but reflex vasodilation due to an increase in metabolic rate. But the tail vasodilation by Isop in the spinal rats can not be explained by reflex vasodilation via the hypothalamus.

References: 1) Fregly MJ (1983) Pharmacology 27:150 -159.

B—28

INORGANIC ION COMPOSITION OF RAIN WATER IN YAKUSHIMA ISLAND

TETSUO ANDOH[1], KAZUYUKI NAKAMATA[2] AND SUMINORI AKIBA[1]
Faculty of Medicine, Department of Public Health, Kagoshima University[1]
and Yakushima Public Health Centre, Kagoshima Prefecture[2]

The samples of rain water on Jan. 13, Feb. 23 and March 7 in 1994 were collected at Anbou district and the three points of Nagata district in Yakushima Island. The measuring of inorganic ions of rain water gave the knowledge of a main characteristic of hydrogen-ion concentrations (pH values) of it. The mean of pH values on March was significantly lower than that on January and February, and that at Nagata district was lower than that at Anbou. Twice out of twelve collections of rain water indicated the lower pH values below 5.0, namely acid rain. The chlorine- and sulfate-ion concemtrations of the samples of Yakushima Island were higher than those of Sakuragaoka-Kagoshima, or urban distric, however, the nitrate-ion ones of them of former were lower than those of latter. From the results, the sulfate-ions of rain water of Yakushima Island may partly originate from China, and the exhaust gas of automobiles on the island may little contribute the nitrate-ions of rain water. The chlorine-ions of rain water of the island may originate mainly from the diffusion of sea water, but partly from volcanic gas of Mt. Shin-dake in Kuchinoerabu Island, 15km from Nagata district, as same as those of Sakuragaoka from Mt. Sakurajima. The hydrogen- and inorganic ions of rain water of Yakushima Island should be continually monitored.

B—29

CLINIBAL EVALUATION OF HEALTH EXAMINATIONS OF JAPANESE WORKERS DISPATCHED TO FOREIGN COUNTRIES

MOTOYUKI FUKUMURA, ATSURO HAMADA, MAKIYO MACHIDA
AND SATORU KOJIMA
Japan Overseas Health Administration Center

In order to evaluate health of Japanese workers dispatched to foreign countries, health exam. were performed on 52 Japanese workers before and after their missions. The study involved 42 men and 10 women (mean age:35±1) dispatched to foreign countries for an average of one year. Their missions were in both developing countries(39 subjects) and developed countries(13 subjects). The developing countries included Indonesia(11 subjects), China (8), Singapore (4) etc. The developed countries were the U.S.A. (9), Germany (2) and England (2).

We perfomed the health exam. immediately before and after the workers' stays. The exam. were: standard hematoserological tests, urinalysis, ChestXP, electrocardio-gram(ECG), blood pressure measurement, gastrointestinal XP, and abdominal ECHO.

Participants were also interviewed before and after their missions regarding their life styles including diet, amount of sleep, and physical exam.

Results of the tests taken after the workers' return were compared with results prior to their departure.

The lifestyles(diet, sleep, and physical exercise) of the workers changed significantly during their stays abroad. The changes were more marked in the workers dispatched to developed countries than those dispatched to developing countries.

No significant difference were found in laboratory data-including liver function and serum lipid, blood pressure, and body weight-before and after their stays. Although health problems were not detected in the workers whose lifestyles changed significantly, it may nevertheless be necessary to educate the workers in how to maintain healthy lifestyle in order to prevent health problems caused by changes in environment and life-

style.

B—30

BACTERIOLOGICAL ANALYSIS OF STOOL FOR 5 YEARS IN KAGOSHIMA AIRPORT QUARANTINE STATION

SATORU HAGIO AND BISHO SAKUMOTO
Kagoshima Quarantine Station

An attempt to evaluate the local airport quarantine system was done in Kagoshima International Airport. Kagoshima is one of the entrances of south-east Asian countries, where various infectious diseases are endemic. According to the Quarantine law, 51 travelers with diarrheal symptoms by their declaration were checked by quarantine officer for 5 years (1989-1993). Bacteriological examination for their stool was done. 14 positive results were obtained (V. parahaemolyticus=5, Enteroinvasive E. coli=2, P. shigelloides=6, and Salmonella=1). No V. cholerae 01 was found. In the same period, 3 cholera, 9 dysentery and 1 paratyphoid fever patients entered the local infectious disease wards in Kagoshima prefecture. In Kagoshima Airport as a small local international airport, quarantine system is not functioning well. Even in local airport, health consultation and adequate privacy-oriented information service of tropical medicine for the travelers are necessary. Amendment of Quarantine law and Prevention of infectious disease law is recommended for the more effective control of imported infectious disease.

B—31

ASYMPTOMATIC INFECTION OF *CRYPTOSPORIDIUM MURIS* IN HUMANS

TATSUYA KATSUMATA[2], DANIEL HOSEA[1], EDDY BAGUS WASITO[1], PITONO SOEPARTO[1], IGN GDE RANUH[1] SHINJI YOSHIKAWA[2], MITSUO KAKU[2], SHIGERU KOHNO[2] AND KOHEI HARA[2]
Tropical Disease Research Center, Medical School, Airlangga University[1]
and Second Department of Internal Medicine, Nagasaki University School of Medicine[2]

In mammals, *Cryptosporidium parvum* and *Cryptosporidium muris* are accepted as distinct and valid species. The substantial differences in oocyst size as well as location allow the two species of *Cryptosporidium* infectious for mammalian hosts to be distinguished. While *C. parvum* has been recognized as a cause of diarrhea in humans, infection by the parasite, *C. muris* has never been reported in humans.

Of 6328 subjects examined for *Cryptosporidium* in Surabaya, Indonesia, two were excreting oocysts much larger than those of *C. parvum.* and these were morphologically identified as oocysts of *C. muris.* The patients were a 4-year-old and a 6-year-old healthy female children. More than 10^4 oocysts per 1g of feces were found by Sheather's sugar flotation combined with phase-contrast microscopy and modified Kinyouin acid -fast stain. Daily examination revealed that oocyst excretion continued for the next 5 and 6 days respectively without abdominal symptoms. In our study group, the prevalence of *C. muris* infection (0.03%) was lower than that of *C. parvum* infection (1.4%). Both cases infected with *C. muris* were found in rainy season when *C. parvum* infection was more prevalent also.

The present cases showed that *C. muris* can infect humans. Natural infection with *C. muris* has been reported only in rodents and ruminants, while *C. muris* can be experimentally transmitted to mice, rat, guinea pigs, rabbits, dogs, and cats. Although human *C. muris* infection seems to be rare and asymptomatic, the infection might cause some positive symptoms in immunosup-

pressed subjects. Further studies on the source of transmission, location of the parasite, pathogenicity, and epidemiology of *C. muris* infection in humans are needed.

B—32

VENOMOUS SNAKEBITES IN OKINAWA PREFECTURE IN 1993

SEIKI KATSUREN and MASATOSHI NOZAKI
Habu Study Section, Okinawa Prefectural Institute of Health and Environment.

During the year 1993, 159 venomous snakebites were reported in Okinawa Prefecture. 102 bites were caused by Habu (*Trimeresurus flavoviridis*), 40 cases by Sakishimahabu (*Trimeresurus elegans*) and 17 cases by Himehabu (*Ovophis okinavensis*). There were no fatalities reported.

Venemous snakebite usually takes place in early summer and autoumn. In 1993, it was reported that there were 22 cases in October, 16 cases in November and 10 cases in May.

In the case of Habu bites 32 incidents (32%) took place in agricultural fields, followed by 20 incidents (20%) in domestic dwellings, 14 (14%) indoors, 11 (11%) on grassy lands, 10 (10%) on roads and 3 (3%) in mountains and forests. In the case of Sakishimahabu incidents 17 cases (43%) occurred in the fields the most common location. 6 cases (35%) of Himehabubites, the majority, took place in around domestic dwellings.

Although the Habu is nocturnal, many incidents happened during the daytime rather than evenings. In 1993, many Habu bites took place around noon and 20:00. Many of the daytime Habubite cases occurred in sugarcane fields while farmers were working. In other words, farming activity brought individuals closer to Habu. On the other hand, many of the incidents occurring between 18:00 and 06:00 took place within house hold properties. In other word, Habu invaded human communities.

Due to the advancement of medical techniques, well-provided medical institutes and well-developed transportation enabled the victims to reach the medical care in a short time. Case of death are rarely reported and the number of cases with severe parmanent damage have decreased.

Because of serum disease caused by the use of antivenom, for many victims of Sakishimahabu and Himehabu which are less poisonous than Habu, antivenom has not been used. Even Habu bite cases, antivenom was used for 75% of the victims. In recent years, if the victims suffer only slight symptoms, antivenom is not used so often as it used to be.

B—33

THE NEUTRALIZATION EFFECTS OF HABU-ANTIVENOM AGAINST SNAKE-VENOMS EXTRACTED FROM SNAKES RECENTLY CAPTURED IN OKINAWA ISLAND

MASATOSHI NOZAKI, TAKAO KAMURA AND SEIKI KATSUREN
Habu Study Section, Okinawa Prefectural Institute of Health and Environment

Several kinds of venomous snakes, not indigenous to Okinawa, have been imported to Okinawa for various purposes such as production of alcholic beverages containing the whole corpses of snakes, snake powder production, mongoose and snake fight shows for the tourist indurtry and display purposes.

Some of these snakes which had escaped from amusement sites were recently captured on agricultural land and road sides in the farming areas. The incidents are causing a considerable worry to the nearby communities.

Though it is very difficult to estimate whether these snakes reproduce in Okinawa or not, neutralization tests of venoms and habu-antivenom were carried out against the eventuality of attacks.

Hemorrhagic activity was assayed by intracutaneous

injection into rabbits and intramuscular injection into mice. Lethal toxicity was tested by intravenous injection into mice.

The results were as follows:

1. LD_{50} of snakes recently captured in Okinawa island was as follow: Habu (*T. flavoviridis*) venom: 35.0 (31.8～38.5) μg, Sakishima habu (*T. elegans*) v.: 48.0 (45.9～50.2) μg, hybrid of Habu and Sakishima-habu v.: 21.0 (19.1～23.1) μg, Taiwan-habu (*T. mucrosquamatus*) v.: 30.0 (26.8～33.6) μg, Thai-cobra (*N. kaouthia*) v.: 8.7 (7.9～9.5) μg.

MHD (Minimum Hemorrhagic Dose) of each snake were Habu v.: 0.56 (0.46～0.67) μg, Sakishima-habu v.: 1.80 (1.53～2.07) μg, Hybrid v.: 0.51 (0.41～0.61) μg, and Taiwan-habu v.: 0.56 (0.46～0.67) μg. Thai-cobra v. didn't show hemorrhagic activity.

2. In intravenous injection into mice, 0.1 ml of habu antivenom neutralized lethal activity of 162μg (4.6LD_{50}) of habu v. 99μg (2.1LD_{50}) of Sakishima-habu v. 49μg (2.3LD_{50}) of Hybrid v. and 120μg (4.0LD_{50}) of Taiwan-habu v., but habu antivenom could not neutralize Thai-cobra v. In intramuscular injections into mice, 0.1ml of habu antivenom neutralized hemorrhagic activity of 30μg of Habu v., 30μg of Sakishma habu v., 10μg of Hybrid v. and 30μg of Taiwan-habu v

B—34

STUDY ON CHINESE COBRA (*NAJA ATRA*) TOXOID FROM GUANGXI, CHINA

Y. KAWAMURA[1], A. SAKAI[1], Y. SAWAI[1]
AND N.-P. WAN[2]
Japan Snake Institute, Yabuzuka-honmachi, Nitta-gun, Gunma 379-23, Japan[1]
and Guangxi Medical University, Nanning, Guangxi, China[2]

Detoxification of ethanol fractionated Chinese cobra (*Naja atra*) venom of formalin, and the immunogenicity by the toxoid have been investigated. The venom was supplied by the Guangxi Medical University, Nanning, China. Immunogenicity of the toxoid was tested by injecting the toxoid subcutaneously into guinea pigs. 0.4 or 0.5 ml of antivenom taken from 15 immunized guinea pigs neutralized 20 μg (2 mlds) of cobra venom. Thus, it was calculated that 1ml of the antivenom neutralized 40 or 50 μg (4-5 mlds) of the venom. From the protection test, immunized guinea pigs could survive against intramuscular injection of 0.3 or 0.6 mg (1 or 2 mlds) of the venom. Further, 6 out of 8 guinea pigs survived against the venom of 1.2 or 2.4 mg (4 or 8 mlds).

B—35

COMPARISON ON THE SEASONAL ABUNDANCE OF *ANOPHELES MINIMUS* IN SOUTHERN YUNNAN PROVINCE, CHINA AND NORTHERN THAILAND

MASAHIRO TAKAGI[1], LI-GANG CHE[2], YOSHIO TSUDA[1]
AND WANNAPA SUWONKWERD[3]
Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University[1],
The Research Institute of Malaria Prevention and Treatment of Yunnan Province[2]
and Malaria Center Region 2, Ministry of Public Health, Thai Government[3]

Seasonal abundance of *Anopheles minimus*, one of the important malaria vectors on foothills and forest fringes in southeast Asia was investigated in southern Yunnan Province and in northern Thailand. Night collection for the adults baited by 2 human or 1 baffalo and larval collection by dipping in slow running streams and their surrounding areas were conducted monthly. In Yunnan Province, adults peaked in September, decreased after-

ward, and could not be collected in November. The second peak of adults was encountered in April. In northern Thailand, *An. minimus* was much more abundant than in Yunnan Province, and showed a large peak during the first half of the dry season (November-January). Differences on the seasonal pattern in abundance of the adults may be due to difference in the average temperature between the two study areas. Cool condition during the dry season in Yunnan Province may hinder rapid increase of *An. minimus*, even if conditions of streams such as the water current were stable. This speculation was agreed with results obtained by larval surveys again.

B—36

GROWTH CURVE OF MOSQUITO LARVAL POPULATION AND COMPARISON OF GROWTH RATE

KIYOSHI MAKIYA AND MARIKO NISHIYAMA
Department of Parasitology and Tropical Public Health,
University of Occupational and Environmental Health

It is important to know whether mosquito larval population is young or old in order to decide effective control measure against mosquito-borne diseases. And it is theoretically possible to estimate growth rate of larval populations of mosquito in the field by following successive change of a single age group (cohort) in age (instar) distribution.

However, it is often difficult to follow a single cohort because age distribution of larval populations is overlapped among different cohorts in the field. Cassie's method was introduced to analyze such a polymodal age distribution to follow successive growth of a single larval cohort. Average larval age and standard deviation of the cohort were calculated by simulation program of Cassie's analysis in case of polymodal age distribution. Growth curve of larval population was made by connecting these average larval age serially.

Among several models for organism growth, 3 growth curves, i.e. 1) Logistic, 2) Gompertz and 3) Bertalanffy models were often used for this purpose. Comparison was made among these 3 models to estimate growth rate of larval populations in the field. As a result, Bertalanffy curve, $Lt=Li[1-exp(-k(t-to))]$, was proven to fit best to mosquito larval growth of *Aedes albopictus* and *Tripteroides bambusa*. In this equation, Li is final stage of larval population to be reached and k is growth rate to specify respective curves.

Bertalanffy curves were fitted to 18 *A. albopictus* and 12 *T. bambusa* larval populations surveyed with oviposition traps in a small hill with dense trees. Correlation coefficients showing the curve fitness were significantly high for all the larval populations. Average growth rate (k) was estimated to be 0.104/day for *T. bambusa* and 0.224/day for *A. albopictus*, meaning that *A. albopictus* grew about 2 times as fast as *T. bambusa* larval populations. It became possible to know and to compare growth rate of mosquito larval populations quantitatively by using this growth curve.

B—37

IN VITRO EFFECT OF PLANT-EXTRACTS, ESPECIALLY THOSE IN GUATEMALA, AGAINST *YRYPANOSOMA CRUZI* (MINIREVIEW)

J. MAKI[1], Y. ITO[1], I. TADA[2], K. OGATA[3],
N. GARCIA[4] AND A. CACERES[4]
Kitasato University School of Medicine[1], Faculty of Medicine, Kyushu University[2],
Cooperative JICA Project of Research by Guatemala/Japan for Control of Tropical Diseases[3]
and School of Chemistry and Pharmacy, University of San Carlos, Guatemala

Since Nussenzweig et al.(1953, Rev. Paulista. Med. 42: 57-58) demonstrated the in vitro efficacy of gentian violet on *Trypanosoma cruzi,* the plant-origin drug has been used for the in vitro treatment of the protozoal parasites contained in human blood to be transfused. For the decades, many other medicinal plant-derived drugs have been tested experimentally for their efficacy against *T. cruzi* in vitro. This communication is a minireview of such in vitro studies with special reference to those in Guatemala.

About 20 articles have described the lethal efficacy of medicinal plant extracts against epimastigotes and trypomastigotes of *T. cruzi* in vitro all over the world (Maki & Caceres, 1994, JICA Report, pp.14). In the report, the articles, and names of plants and compounds were presented in a table. To the best of the authors' knowledge, no articles except preliminary reports by Maki & Caceres(1994, ibid.) and Caceres et al.(1995, Jpn. J. Parasit. 44, in press) have been published on the subject in Central American countries, though the countries like Guatemala are rich in medicinal plant resources(Maki & Caceres, 1993, JICA report, pp.28; Caceres et al., 1993, Enfermedades tropicales en Guatemala. Informe Anual 2, 140-143).

One of the newest studies on in vitro efficacy of Guatemalan plant extracts against *T. cruzi*(Maki & Caceres, 1994, ibid.) is described in somewhat more detail as follows. The four kinds of medicinal plants, *Bixa orellana, Neurolaena lobata, Tagetes lucida* and *Smilax regelii* were collected in Mazatenango, Guatemala. The drugs to be tested were extracted from the plants with ethanol and prepared as described in the report(Maki & Caceres, 1993, ibid.). The mixture of 0.1 ml epimastigote suspension in LIT culture medium (Tulahuen strain, 3×10^6/ml) and 0.1 ml extract-LIT medium at the final concentration of 250mg/ml was agitated in small holes of a plate and incubated at 28°C for 48 hours. The negative control was free from any extract. The in vitro efficacy of the crude drugs was compared with that of a reference drug, purified gentian violet at the final concentration of 250μg/ml. After the incubation, 10 μl were aliquoted from each well and put on a glass(Neubauer chamber). The number of parasites was counted under a microscope(×400). The efficacy of gentian violet was confirmed. The comparison of the counted number of parasites in experimental groups with that in the negative control suggested the possibility that the extracts of the four plants were more or less effective against *T. cruzi* epimastigotes under the present condition. Among others, the extract from *N. lobata* was shown to be fairly effective in killing *T. cruzi* in vitro at the significant reduction rate of 60%. The dose-effect relationship in vitro and the possible suppressive effect of the extract on the proliferation of the protozoa in mice are now being studied.

B—38

INFORMATION COLLECTING AND ACCESS SYSTEM OF MEDICAL ASSESSMENT IN THE TROPICS

HIDEYO ITAKURA
Department of Pathology, Institute of Tropical Medicine, Nagasaki University

We as health professionals are constantly asking: 1) how can we keep up-to-date with current information and trends in the tropics? 2) how can we diagnose and treat exotic diseases in the tropics? 3) how can we instantly get access relevant information in the tropics? 4) what therapy should we use in the tropics? 5) where can we go for expert advice in the tropics? Information collecting and access system is a computer-based network and a comprehensive software tool that can help you. It is important to collect information in medical fields in the tropics to perform medical activities with modernized medicine in appropriate methods and ways. The information from tropical areas should be fresh. At the same time, easy access to such information is also important. The plan for establishment of such system in the tropical medicine is under investigation by specialists in various academic fields including epidemiologists, microbiologists, parasitologists, geopathologists, public health specialists, and information technologists. The plan should include: 1) preparation of network, 2) accumulation of products of software, 3) stockpiling of new information and data, and 4) maintenance of the established network. The proposed system should be worldwide and useful to every specialist in medical fields.

B—39

THEME OF HEALTH IN NORTH EAST THAILAND

MICHIYO UMEMOTO[1], JUNKO TATEMATSU[1], KUNIYASU SUZUKI[1],
KENJI YAMAGA[1], TOMOKO KUNII[1], HIROMI SUZUKI[1]
AND YOSHINARI MORIYAMA[2]
Suzuka University of Medical Sciences & Technology[1]
and Teikyo University, Faculty of Medicine[2]

Though, Thailand is one of tropical developing country, recentry the economic growth is remarkable. Life style of rural area was also changed. In North east Thailand, change of health situation and problem of medical subject were studied. Price of groceries were 10% that of Japan except egg which price was same as Japan. Relatively, daily necessaries such as books, pens, papers, etc. were more expensive than groceries. Eating habit was same as 10 years before as far as their eating sticky rice as the staple food. But the divercification of food was also seen in the dish compared with situation of 10 years before, because spread of using money became popular in the village. Though, the season this study was carried out was famous season of epidemic diarrhoea. Outbreak of diarrhea due to bacterial infection was not seen, and geriatric diseases seemed predominant rather than. A lot of females 20 years to 40 years old who diagonized was overweighted girl. The reason why hypper-tension patients were not many was because the costom of little intake of salt. Twelve infant were tested for finding the carrier of pathogenic bacterria. Non-01 *Vibrio cholerae* as isolated from one of them. Numbers of delivery times were 2 times, and this fact suggest that effect of birth control by Thai government had been popular in rural village of North East Thailand.

B—40

ENVIRONMENTAL HEALTH IN NORTH EAST THAILAND

HIROYUKI MASUDA[1], KAZUYA HATA[1], KEN OSUMI[1],
RIKI KUWABARA[1], EIKO YAMANAMI[1], MAKIKO MURAMATSU[1],
HARUMI ITO[1] AND YOSHINARI MORIYAMA
Suzuka University of Medical Sciences & Technology[1]
and Teikyo University, Faculty of Medicine[2]

Construction of toilets and preparing big jar for stocking rain water of drinking purpose for dry season were major sanitary project in Thailand. Frequency of drinking under ground water became less than before. On the other hand, because the cutting forest, the climate changed to dry condition and salt soil from underground increase the concentration of NaCl%. Environmental research of a rural village in Northeast Thailand had done in March 1994 dry season for this study. Concentration of NaCl in water both drinking and domestic, bacterial contamination of water by coliforms, fishes and domestic animals by salmonella were studied in this research. Though, ten years before, people in the village used rain water in rainy season and shallow well in dry season for drinking purpose at the time when last reaseach had done in this village, most of hausing equipped a large jar and they used rain water as drinking water even in dry season in this research. Majority of hause in the village had refrigerator and, drinking water was usually colled. Most of drinking water was contamined by coliforms, because chlorination had not done. *Vibrio alginolyticus* was isolated from fresh water fishes in pond of this village which showed a little NaCl concentration. Non-01 *V. cholerae* and *V. fulvialis* were isolated from a duck in the village. As mentioned above, especially system of drinking water shows, circumstance of Northeast Thailand improved recentry, and still have problems of Vibrio contamination due to high NaCl concentration of water.

B—41

GENETIC POLYMORPHISM OF GROUP-SPECIFIC COMPONENT, TRANSFERRIN AND ALPHA-1-ANTITRYPSIN IN KAGOSHIMA PREFECTURE: THE SEROLOGICAL CHARACTERS OF THE POPULATIONS IN THE NANSEI-SHOTO

KAZUTOSHI AGO AND OSAO TSUGANEZAWA
Department of Legal Medicine, Faculty of Medicine, Kagoshima University

Distribution of group-specific component(GC), transferrin(TF) and alpha-1-antitrypsin(PI) variants were investigated to clarify characters on serological genetics of Mainland, Tanegashima, Yakushima and Amami Oshima populations in Kagoshima Prefecture. Tanegashima, Yakushima and Amami Oshima are islands in the Nansei-Shoto. Typing was performed by isoelectric focusing with acrylamide gels followed by immunoprint for GC and protein staining for TF and PI.
【GC types】 Geographical cline of GC*1F, GC*1S and GC*2 frequencies in Japan was examined by use of data estimated in this study and 23 data which had been obtained at 17 districts. Existence of geographical cline that the GC*2 frequency gradually increased and GC*1S frequency decreased from Aomori to Okinawa was revealed. Six different types of rare variants were observed, and the distributions of the variants were not significantly different among the four populations.
【TF types】 The frequencies of common alleles(TF*C1, TF*C2) in Mainland, Tanegashima, Yakushima and Amami Oshima were within range of the values of which the allele frequencies had been obtained at 12 districts in Japan. A new variant with a band which more cathodically located than the Dchi band was observed in the populations of Amami Oshima and Yakushima. This variant was tentatively designated Dama. The TF*Dama frequencies were 0.0055 in Amami Oshima and 0.0010 in Yakushima. From these findings, the Dama is likely to be characteristic of the population in Amami Oshima.

【PI types】 The frequencies of common allele (PI*M1, PI*M2 and PI*M3) in Mainland, Tanegashima, Yakushima and Amami Oshima were within range of the values of which the allele frequencies had been obtained at 10 districts in Japan. Interesting two rare variants which were tentatively designated Eama and Pya1 were found in this study. The Eama was restrictively observed in the population of Amami Oshima only (PI*Eama frequency: 0.0063) and the Pya1 in the population of Yakushima only(PI*Pya1 frequency: 0.0059). The Eama was identified with I by the comparison test for reference serum. The I is extremely rare in the other Japanese populations so far investigated. From these findings, the Eama and the Pya1 are likely to be characteristic of the populations in Amami Oshima and Yakushima respectively.

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