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Editorial

Present Situation and Future Prospects of Tropical Medicine in Developed Countries

During the past decade international relationships have been strained by a variety of problems, not only diplomatic and economic but also ethnical and religious. The field of tropical medicine is no exception. There are overwhelming and vitally important health problems in tropical areas located for the most part in developing countries. It is essential that the relevant institutions in European and American countries, whose history of some one hundred years is marked by distinguished achievements in the protection of human health in the tropics, give a thought to what is going on and what is necessary in the field of modern tropical medicine. The health of the developing world should always be in the consciousness of the developed industrialised countries.

The symposium, entitled "Present situation and future prospects of institutions of tropical medicine in advanced countries", was held in November, 1992, as part of the activities to celebrate the 50th anniversary of the Institute of tropical Medicine, Nagasaki University (Nagasaki Institute) (1992). The symposium was organized by the Nagasaki Institute and the Japanese Society of Tropical Medicine, and supported by the Ministry of Education, Science and Culture, Government of Japan. Participating institutions were Bernhard Nocht Institute for Tropical Medicine, Hamburg (1990), Prince Leopold Institute of Tropical Medicine, Antwerpen (1990-1991), Liverpool School of Tropical Medicine, Liverpool (1990-91, 1991-92), National Institute of Allergy and Infectious Diseases, NIH, Bethesda (1988, 1992), John E. Fogarty International Center, NIH, Bethesda (1992), Tulane University School of Public Health and Tropical Medicine, New Orleans, TDR, WHO, Geneva (1990, 1991), and International Health Institute, Case Western Reserve University, Cleveland. The annual and research reports of the London School of Hygiene and Tropical Medicine, London (1990-91, 1991) were sent to the organizers.

The Nagasaki Institute is the only government collaboration institute in the field of tropical medicine in Japan. During the fifty years since its inauguration, the institute has devoted itself to research and education in the field of endemic and tropical medicine and to the exchange of scientific and technological knowledge through medical cooperation, both in Japan and abroad. Looking back over the history of the past fifty years, the endeavors of the institute have been promoted by its domestic and international research colleagues in related academic fields.

There are a lot of topics and problems concerning institutions of tropical medicine. Some of them were discussed at the meeting.

—*Principal function*: The principal function of the institution is the sponsorship and organization of research and education on tropical medicine. Additional functions include the dissemination and exchange of information, the promotion of cooperation among institutions and other appropriate scientific activities.

—*Organization and infrastructure*: The organization and infrastructure may differ from institute to institute depending upon respective circumstances. Some institutes operate field stations in the tropics where epidemiologic, patient-oriented and laboratory investigations are being conducted. There is always a need for patient care, with the mission to diagnose and treat patients with infectious tropical diseases. In addition there should be the clinical division with beds and an ambulatory facility for patient studies and care. Collaboration with local hospitals in the tropics is important. There is often a shortage of staff of intermediate level as medical doctors and practicing nurses. It is difficult to find young staff members, especially physicians, as research fields are too specialized and of limited importance in industrialized countries. Medical research in developing countries still lags behind because of the lack of means, and of research staff and technicians.

The Nagasaki Institute is affiliated with Nagasaki University and is under government control by annual budget. But the staff limitations in small laboratories and the necessity of going abroad often disturb the continuity of laboratory research.

—*Research activities*: The expanded definition of tropical medicine and recent scientific advances in the field have resulted in a broadening of basic scientific policies and research programs, and in a demand for the participation of molecular biologists and modern laboratory

techniques. Emphasis is placed on disease-oriented basic research; disease mechanisms, pathogenicity of infectious agents, immune response of the host, host-parasite relationships, vaccine development, etc. To this purpose, a section for molecular biology should be established through which the technologies of molecular genetics, immunology and protein chemistry should be introduced. There is a need for a continuing, active infrastructure for the organization of basic sciences, not to mention a well-defined method for the co-ordination and exchange of information.

Attention must also be paid to the enhancement of fieldwork and health care activities: for example, epidemiology, methodology of protection of various kinds of tropical diseases, environmental physiology, geographical and ethnological pathology, comparative studies on diseases between the tropics and the temperate areas, and interdisciplinary research on the relationships among environmental factors, human ecology and disease manifestations. The full impact of health related factors can be examined only through community studies.

One must take care not to compete with universities. One works essentially in the field of applied research trying to use the technological progress of medicine to improve the health of the third world, but the border with basic research can be hazy.

—*Education, teaching program, training system*: Teaching in the field of imported diseases is the task of a specialized institute. Educational programs should be designed so that each academic laboratory achieves its own objective in the field of expertise. They are; 1) lectures and practicals for undergraduate medical students at universities, 2) a postgraduate course leading to Ph. D. or other degrees, 3) participation in a training courses in tropical medicine for doctors, nurses, technologists and relevant professionals, and 4) medical research training courses for researchers from abroad or in the tropics supported by government or private organizations.

—*Academic services*: In addition to the programs mentioned above, routine academic services of the Nagasaki Institute include laboratory diagnostic examination of viral, bacterial, protozoal and parasitic diseases, postmortem examinations and histological diagnostic pathology of surgical specimens, and the clinical treatment of patients with infectious diseases at University Hospital.

—*Collaboration with other organizations*: Considerable emphasis has been placed on collaboratory work of many kinds. Two or more institute-wide research programs are necessary. They constitute the main research effort in which major personnel and finacial resources are tied up. The collaboration at the level of the universities should be within the scope of the institute. The aim should be to achieve a critical mass of talent in these programs to assure productivity and success. Widely expanding international collaborations are important.

—*Budget and research funds*: Research funding is an important matter. Appropriate official funding for research activities in tropical countries is necessary. Funding should be reserved for development activities abroard or for research activities in the country itself. Each research scientist should have the opportunity to receive research funding from all areas of society, both public and private. The annual budget for the management of the Nagasaki Institute is provided by the government through the university.

—*Laboratory facilities*: Laboratory facilities including special facilities for tropical medicine should be available on the basis of the budget and research funding.

—*Scope and future policy*: One of the responsibilities of the institution of tropical medicine should be to provide leadership and direction in the advancement of tropical medicine and primary health care and to perform other appropriate duties as required. The institution should be responsible for the preparation and up-dating of information on tropical medicine. An important social contribution is the practical work conducted in tropical areas according to the social needs and policy of countries concerned. With regard to the strategy for the development of modern tropical medicine, it is important to; 1) encourage the formation of international cooperation programs such as activities under the ODA program, 2) establish relationships with international organizations such as WHO, 3) facilitate the transfer of research knowledge, equipment and its application to people in the tropics and provide help and appropriate assistance to the developing countries, 4) disseminate information about opportunities for education and training in tropical medicine, and 5) give advice to relevant government organizations based upon our knowledge and experience. We must seek to influence politicians and the public on the importance and justice of tropical medicine for those we serve in the developing countries.

As a whole, the Nagasaki Institute is on safe ground in Japan for the time being. However, its survival and future will depend on its capacity to adapt to the largely unforesseable evolution of the next decennial and on its ability to define clearly its place in research and educa-

tion not only in the tropical developing countries but also in economically advanced countries. On the basis of our commitment to tropical medicine and to international friendship, we intend to continue our collaboration with developing countries and advanced countries. We hope that the above mentioned symposium will be the first of many future events in Japan attended by speakers from institutions of tropical countries in the near future.

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A NEW BLACKFLY SPECIES OF *MAYACNEPHIA* FROM GUATEMALA (DIPTERA: SIMULIIDAE)

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Received November 18 1992/Accepted December 16 1992

Abstract: A new blackfly species, *Mayacnephia tadai* sp. nov. was described based on the female, pupal and larval specimens collected from a seasonal small stream in upland of Guatemala. This species is readily distinguished from the other known species by the number of pupal gill filaments (six).

Mayacnephia is a small genus of the family Simuliidae consisting of 10 species, most of which were reported from central America (Wygodzinsky and Coscarón, 1973). Recently we collected an additional species belonging to this genus from an upland stream in Guatemala which is easily separated from the known *Mayacnephia* species. This is described as a new species below. The taxonomic characters and their terminology used here follow those of Wygodzinsky and Coscarón (1973).

DESCRIPTION

Mayacnephia tadai Ochoa and Takaoka, new species

Female. Body length 3.0 mm. *Head* narrower than thorax. Frons dark brown, covered densely with whitish scale-like pubescence; frons (Fig. 1) narrow with frontal angle 60 degrees. Fronto-ocular areas (Fig. 1) well developed. Clypeus dark brown, covered densely with whitish scale-like pubescence, interspersed with several dark long hairs. Antenna consisting of 2+9 segments, dark brown; 1st flagellomere elongated, ca. 1.8× length of 2nd flagellomere. Maxillary palp with 3rd segment (Fig. 2) of moderate size, subequal to or slightly longer than 4th segment; sensory vesicle (Fig. 2) oblong, ca. 0.43× length of 3rd segment. Maxilla with 9 or 12 inner teeth and 13 or 14 outer teeth. Mandible with ca. 30 inner teeth and 12 outer ones. Cibarium unarmed. *Thorax.* Scutum dark brown, densely covered with recumbent whitish scale-like pubescence. Scutellum brown covered densely with whitish scale-like pubescence and with several dark upstanding hairs. Postscutellum dark brown, bare. Pleural membrane bare. Katepisternum glabrous, in profile as long as high, and bare. *Wing lost.* Legs also lost except for all coxae, fore femur and hind basitarsus; hind basitarsus (Fig. 3) nearly parallel-sided, brown, and with well developed calcipala. *Abdomen.* Basal scale dark brown with dark and pale hair fringe; dorsal surface of abdomen dark brown, densely covered with recumbent whitish scale-like pubescence; tergites of posterior segments semi-shiny. *Terminalia* (Figs. 4 and 5). Sternal plate of 7th abdominal segment well developed, large and with numerous hairs. Sternal plate of 8th segment well developed, widely bare medially but with ca. 12 hairs on each side; anterior gonapophyses large, subtriangular, apices rounded, medial borders approximated, surface with numerous microtrichia and with 15-18 setae. Genital fork inverted-Y shaped, stem and part of arms heavily sclerotized, and arms with distinct projection directed anterodorsally. Paraproct in ventral view slightly shorter than wide, and with ca. 9 dark hairs; in lateral view paraproct not produced posteriorly under cercus. Cercus in lateral view semicircular in shape, and with numerous hairs. Spermatheca large, well sclerotized except small circular membraneous area at base of spermathecal duct, and without internal setae.

Pupa. Body length 4.0 mm. *Head* and *thorax*. Integument yellowish and moderately covered with minute tubercles. Head with 2+2 frontal and 1+1 facial trichomes; thorax with 5+5 trichomes, all simple and slender. Gill (Fig. 6) consisting of 6 tubular filaments in pairs; all filaments shortly stalked, subequal to each other in length and thickness, diverged widely and each

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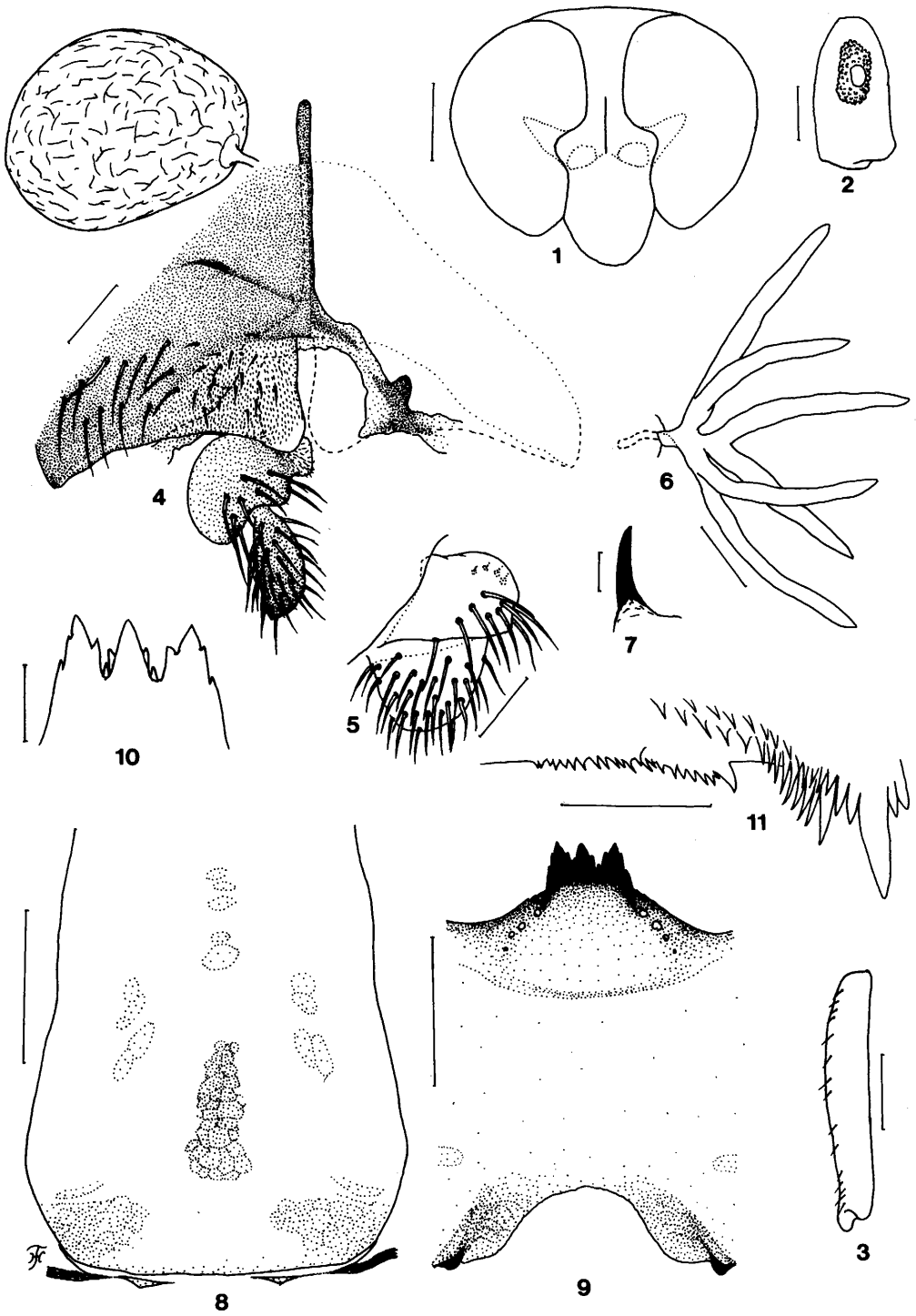

Figure 1-11 *Mayacnephia tadai* sp. nov. 1, female head showing narrow frons; 2, 3rd segment of female maxillary palp showing sensory vesicle inside; 3, basitarsus of female hind leg; 4, female terminalia in situ (ventral view) showing 8th sternite, anterior gonapophyses, genital fork, paraproct, cercus and spermatheca; 5, paraproct and cercus in lateral view; 6, pupal gill filaments (lateral view); 7, terminal hook of pupal 9th abdomen (lateral view); 8, cephalic apotome and cervical sclerites of larval head; 9, ventral surface of larval head capsule showing hypostomium and shallow postgenal cleft; 10, apical tip of larval hypostomium; 11, apical tip of larval mandible. Scale bars, 0.2 mm for Figs. 1, 3, 8 and 9; 0.5 mm for Figs. 2, 4, 5, 6 and 10; 0.05 mm for Figs. 7 and 11.

with tapered apex; surface of filaments smooth. *Abdomen.* All terga tuberculate; terga III and IV each with 4+4 hooks posteriorly; all hooks simple; terga V-IX each with continuous spine-combs anteriorly; terminal hooks (Fig. 7) well developed and elongated. All sterna except last one tuberculate; sterna V I and V II divided longitudinally along middle by membraneous, striate area; sterna V-V II with 4+4, 3+3 and 2+2 simple hooks, respectively, accompanied by 2+2 hooks in pleural membranes; 9th segment ventrally and laterally with several curved, apically coiled hooks. *Cocoon.* small, short, slipper-shaped, rather loosely woven anteriorly, and covering the pupal abdomen alone.

Larva. Body length 6.5 mm. Body color pale yellow. Cephalic apotome (Fig. 8) broadest well before posterior border, and almost pale with posteromedian and posterolateral head spots yellow. Cervical sclerites (Fig. 8) small, narrowly fused to upper ends of postocciput. Antenna as long as stem of cephalic fans; length ratio of 1st, 2nd and 3rd segment 1.0:0.85:1.1. Cephalic fan with 36 main rays. Mandible (Fig. 11) with numerous mandibular serrations. Hypostomium (Figs. 9 and 10) with 13 apical teeth in 3 conspicuous groups, with median tooth and corner teeth the largest; lateral borders weakly serrated anteriorly; hypostomial setae 4 in number, diverging posteriorly from lateral borders. Postgenal cleft (Fig. 9) small, ca. 3× length of postgenal bridge. Thoracic and abdominal cuticle bare except colorless simple setae at base of anal sclerite. Anal gill lobes simple. Anal sclerite X-formed, posterior arms ca. 1.8× as long as anterior arms. Ventral papillae present. Posterior circlet with ca. 76 rows of up to 18 hooklets per row.

Type specimens. Holotype, reared adult female mounted on glass slide, together with its associated pupal skin and cocoon, Rio Amates, Guanagazapa, Escuintla, GUATEMALA, 23. VI. 1986. J.O. Ochoa; paratypes, 1 pupa in alcohol and 1 larva on slide, same data and date as holotype. All these type specimens will be in due course deposited in the British Museum (Natural History), in London.

Ecological notes. These pupae and larva were collected together with *M. aguirrei* (Dalmat) from twigs in a small temporary stream (width 50 cm; water discharge 8 liters/sec.; altitude ca. 1,000 m above sea level). Female has the well developed, toothed mandibles and maxillae but its feeding habit is unknown.

Distribution. Guatemala.

Remarks. This new species was named after Dr. Isao Tada, professor of Kyushu University, Japan, in recognition of his great contribution of research and control of Guatemalan onchocerciasis.

The number of samples examined was small and even the unique female adult specimen reared from the pupa was not in a normal condition, missing the wings and most parts of legs. Nonetheless, this species was easily assigned to the genus *Mayacnephia*, defined by Wygodzinsky and Coscarón (1973), by a combination of the diagnostic characters of the pupa and larva, such as pupal cephalic sclerite with 2+2 frontal trichomes and tubular gill filaments, and larval hypostomium with 13 apical teeth in three groups.

This genus is mainly distributed in central America extending northerly to Canada and southerly to Venezuela and contains 10 species (Crosskey, 1988), all of which are so similar to one another in the adult and larval stages but differ remarkably in the pupal stage. The female and larva of the present new species also are very similar to those known species. However, the number and shape of the pupal gill filaments easily separate this species from the others.

From Guatemala, prior to this new species, three *Mayacnephia* species, namely *M. aguirrei, M. pachecolunai* (de Leon) and *M. roblesi* (de Leon), have been known, all of which have the differnt shapes of the pupal gills (Dalmat, 1955).

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THE EPIDEMIOLOGY AND CONTROL OF MALARIA IN THE PHILIPPINES*

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Abstract: Malaria has always been considered a major public health problem in the Philippines. From 1926-1946, malaria was identified as the leading cause of mortality and morbidity having at least two million cases with from 10,000 to 20,000 deaths annually (Russel, 1936). The implementation of a nationwide malaria control/eradication program starting 1953, brought down the level of malaria so that it is no longer among the 10 leading causes of mortality. At present, it is still the 10th leading cause of morbidity. In 1991, the malaria endemic population is about 12.0 out of the total 64.5 million. It is endemic in 72 out of 75 provinces in varying endemicity ranging from an Annual Parasite Incidence (API) of 0.62 to 43.8/1,000 population. Malaria is hypoendemic along the coastal and plain areas and hyperendemic in the foothills and forest fringes. There is generally no transmission in cities, urban areas and big centers of population because of unfavorable breeding condition of the vector. Practically, no transmission is found in areas 3,000 ft above sea level.

The main mosquito vector, *Anopheles flavirostris*, breeds in slow flowing clear, partly shaded streams. The secondary vectors are *An. litoralis*, a brackish water breeder; *An. balabacencis*, which are mostly confined to forested areas; *An. mangyanus*, which has the same habitat as the *An. flavirostris*, but thrives better in forested or heavily vegetated areas and *An. maculatus* which prefer clear flowing mountain streams exposed to sunlight.

The most common parasite species is *Plasmodium falciparum* (66%) followed by *P. vivax* (33%) per 1991 records. *P. malariae* is rare and *P. ovale* was reported only once in 1969. The results of 18 years monitoring of drug resistant *P. falciparum* to chloroquine varies from 28 to 89% classified as RI with some RII level. In the years 1982, 1985 and 1987, 100% *P. falciparum* resistance (RI) to amodiaquine have been recorded.

The Malaria Control Program objective is to reduce the incidence of malaria to 1/1,000 population by year 2,000 and prevent its reintroduction to malaria freed areas. The key strategy is to implement effective vector control measures in areas with API>5/1,000 population complemented by the rational use of appropriate anti-malaria drugs. In areas with API < 5/1,000, intensified case finding and immediate treatment thru PHC supplemented by an active health information system directed towards personal protection and prevention from malaria. Following these strategies, the national API was reduced from 14.5/1,000 in 1987 to 7.3/1,000 population ending 1991.

INTRODUCTION

Malaria has always been considered a major public health and socio-economic problem in the Philippines. From 1926-1946, malaria was identified as the leading cause of mortality and morbidity having at least two million cases with from 10,000 to 20,000 deaths annually (Russel, 1936). It has been a deterrent in socio-economic development as it has affected resettlement programs, roads, and bridge constructions, agriculture development and forest conservation. Various economists estimated that the government's loss due to malaria ranged from 100 to 750 million persos annually (Ejercito, 1954), when the dollar-peso rate was 1:2. The current exchange rate now is 1:26.

EPIDEMIOLOGY

1. General epidemiology and distribution

Malaria is widely distributed in the Philippines. At present, it is endemic in 72 out of 75 provinces with varying degrees of endemicity ranging from an Annual

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*Presented at Research Coordination Meeting between Endemic Countries and Non-Endemic Countries on Important Tropical Parasitic Diseases, held at Maebashi, Japan, July 1992.

Parasite Incidence (API) of 0.62 to 43.8/1,000 population. In 1991, the endemic population is 12.0 out of the total 64.5 million. Malaria is hypoendemic along the coastal and plain areas and becomes hyperendemic as one enters the foothills and forest fringes. It is generally unstable with tendency to develop into epidemics. Transmission is more persistent in the difficult forest fringes because of the presence of tribal moving minorities and illegal settlers not normally reached by health services. Transmission is associated with the abundance of breeding places and density of the principlal vector, *An. flavirostris* and is perennial with two distinct peaks, at the beginning and end of the rainy season, and generally lower during the hot summer months. The temperature ranging from 20-34°C is favorable for perennial transmission of *P. vivax* and *P. falciparum* while rainfall and humidity conditions are ideal for vector breeding and long survival. Although most provinces are endemic, there is no transmission in cities, urban areas and big centers of population because of unfavorable breeding condition of the vector. There is also no transmission in areas 3,000 ft above sea level. The provinces of Cebu, Leyte and Catanduanes are malaria-free.

2. Mortality and Morbidity

Malaria was already present among the natives when Magellan first landed on the islands in 1521 (Russel, 1936). Since then, it remained a problem.

From 1946 to 1952, the Department of Health has reported that malaria was the leading cause of morbidity and the 5th cause of mortality. Due to control/eradication efforts, morbidity rates went down from 1,000.7/100,000 to 100.8/100,000 while mortality likewise dropped from 91.0 to only 4.1/100,000 from 1946 to 1966. In 1989 morbidity rate is at 208/100,000 and mortality rate is down to 1.6/100,000. At present, malaria is no longer among the 20 leading causes of mortality and only the 10th leading causes of morbidity (Table 1).

3. Entomology: The vectors and its bionomics

So far, 34 species of *Anopheles* in the Philippines have been confirmed by local entomologists individually or in collaboration with foreign workers.

The primary vector of malaria in the Philippines is *An. flavirostris* (Ludlow). The secondary vectors are: *An. litoralis* (King), *An. balabacencis* (Baisas), *An. mangganus* (Banks) and *An. maculatus* (Theobald).

3.1 *An. flavirostris* is widely distributed throughout the islands compared to the 4 secondary vectors which are limited in distribution because of breeding and behavioral characteristics. *An.*

Table 1 Malaria morbidity and mortality statistics from 1946-1989 per 100,000 population

	Year	Population	Morbidity		Mortality	
			Number	Rate	Number	Rate
	1946	18,434,400	184,482	1000.7	16,783	91.0
	1947	16,785,700	119,395	635.6	12,070	64.3
	1948	19,143,800	85,732	447.8	10.558	55.2
	1949	19,689,800	70,283	357.0	8,801	44.7
	1950	20,315,800	63,075	310.5	7,778	38.3
	1951	20,962,800	54,142	258.3	7,721	38.8
	1952	21,628,300	54,591	252.4	7,170	33.2
	1953	22,316,000	54,119	242.5	6,720	30.1
	1954	23,025,500	71,363	309.9	5,236	22.7
	1955	23,747,600	79,707	335.5	3,714	15.6
	1956	24,513,000	73,560	300.1	2,804	11.4
	1957	25,292,400	60,029	237.3	2,376	9.4
	1958	26,096,600	71,666	274.6	2,253	8.6
	1959	26,926,400	61,645	228.9	1,763	6.6
	1960	27,792,000	55,252	198.8	1,587	5.7
	1961	28,727,000	44,546	155.1	1,373	4.8
first decentralization	1962	29,698,000	40,342	135.8	1,273	4.3
	1963	30,709,000	36,295	118.2	1,114	3.6
	1964	31,270,000	40,854	130.6	976	3.1
	1965	32,345,000	28,988	89.6	1,015	3.1
	1966	33,477,000	33,737	100.8	1,373	4.1
	1967	34,656,000	31,441	90.7	1,147	3.3
	1968	35,003,000	28,354	79.0	1,061	3.0
	1969	37,158,000	31,756	85.5	860	2.3
	1970	36,849,000	28,594	77.6	666	1.8
	1971	37,959,000	25,338	66.8	547	1.4
	1972	39,040,100	27,090	69.4	656	1.7
	1973	40,219,000	31,999	76.6	845	2.1
	1974	41,457,100	27,420	66.1	938	2.3
	1975	42,517,300	27,077	63.7	1,018	2.4
	1976	43,751,300	35,553	81.3	997	2.3
	1977	45,005,300	29,955	66.6	974	2.2
	1978	45,528,500	35,353	77.7	1,077	2.4
	1979	46,580,400	31,779	68.2	1,142	2.5
	1980	48,316,503	39,678	82.1	1,091	2.2
	1981	49,536,022	44,118	89.1	1,071	2.2
	1982	50,783,065	40,496	79.7	985	1.9
	1983	51,973,651	55,019	105.9	1,086	2.1
	1984	53,192,708	107,485	202.1	923	1.7
	1985	54,668,332	121,975	223.1	1,166	2.1
second decentralization	1986	56,004,130	124,153	221.7	1,156	2.1
	1987	57,356,042	121,097	221.1	1,226	2.1
	1988	59,721,307	114,679	195.3	1,176	2.0
	1989	60,096,988	125,114	208.0	986	1.6

From Philippine Health Statistics: 1946-1989

flavirostris breeds in slow flowing, clear, partly shaded streams and seepages which abound in rolling foothill areas. It is generally zoophilic. It has been considered a wild species and is seldom found resting inside houses. When it enters a house it prefers resting on walls and objects within the 0-2 ft level from the floor, and around corners with 0-5 candle power light intensity. *An. flavirostris* is readily recognized by a golden flaviscence on the ventral apical half of the proboscis. *An. flavirostris* is still susceptible to DDT.

3. 2 *An. litoralis* thrives in brackish water, lagoons, rock pools and crevices on coral reefs. This species is very well adapted to sea water of high salinity. *An. litoralis* has been found to be a major vector in the Sulu Archipelago and the coastal areas of Western Mindanao. It resembles *An. sundaicus* except for the presence of the prehumeral pale spot on the wing. Adults may be found indoors but prefer to bite outside houses.

3. 3 *An. balabacencis* is named after the small island of Balabac in the southern tip of Palawan. It was thought to be a complex form of *An. dirus* but Peyton and Harrison in 1979 confirmed it to be different and thus retained its name of *An. balabacensis*. It breeds in forest rainpools, dug wells, water in logging roads, tire prints and animal wallows. It is highly anthropophilic but also feeds on monkeys thereby serving as a vector of simian malaria.

3. 4 *An. mangyanus* is an indigenous Philippine species named after the Mangyan native tribe of Mindoro. It has the same breeding habitat as that of *An. flavirostris* but thrives better in forests or semi-forested areas. The adult resemble *An. flavirostris* except for the absence of the flaviscence on the proboscis and the presence of two pale spots on the basal third of the coastal vein. It is exophilic but tends to be anthropophilic in the absence of available host preference in developed areas.

3. 5 *An. maculatus* is exophilic, strongly zoophilic, seldom enters houses and prefers to rest outdoors amongst vegetations. It breeds in clear flowing mountain streams exposed to sunlight but not in forested areas. Ejercito *et al.* (1934), found the sporozoites in the salivary gland and since then subsequent dissections failed to show its presence.

4. Parasitology and drug resistance problem

The most common parasite species is *P. falciparum* with around 66% followed by *P. vivax* with about 33% per 1991 records. In the past 30 years, the falciparum/vivax ratio varies from 60-70% in favor of falciparum. *P. malariae* is rare and *P. ovale* is reported only once in Palawan in 1969.

4. 1 Drug resistance problem

Resistance to anti-malaria drugs was first reported when a malaria technician stationed in Palawan in 1968 got malaria and did not respond to two courses of amodiaquine treatment. The occurence was traced to contract workers returning from abroad. Three foci of drug resistance were subseqently identified: one in Palawan, another in Aklan, Visayan Island group and the third was in the Central Luzon. From that time on, monitoring for drug resistance to malaria was pursued nationally through both macro and microtests. The results of 18 years survey by the Malaria Control Service from 1974 to 1991 (macro and micro *in vitro* techniques) varies from 28 to 89% resistance to chloroquine, mostly RI and RII levels (Table 2). Since 1988 to 1990 however, micro *in vitro* tests reveal a 100% sensitivity to chloroquine. From 1982 to 1987, 100% *P. falciparum* resistance (RI) to amodiaquine have been recorded. Multi-drug resistance has been found in Sulfadoxine-pyrimethamine. For the past five years, Mefloquine and Quinine remain sensitive (Table 2).

4. 2 Micro test kits and incubator productions for drug resistance monitoring

The Malaria Control Service, Philippines, in coopperation with WHO/CIDA is producing Drug Resistant Micro Test Kits A and B and portable incubator for world distribution. Supplies for these test kits and incubators can be ordered through WHO, Western Pacific Region, Manila.

CONTROL OF MALARIA

1. Historical background and program development

Malaria control efforts started in 1926 when a Malaria Control Section was organized in the Bureau of Health. Selective larviciding with Paris Green was initiated and quinine and plasmochine were used for chemotherapy.

By 1930, naturalistic and mechanical methods were used such as damming, periodic flushing (automatic siphons), exposure of breeding streams to sunlight,

Table 2 *In vitro* studies on drug resistance, Malaria Control Service/Philippines, 1974-1990

Year reference	Method	Respose					Place of study
		Amodiaquine	Chloroquine	Mefloquine	Quinine	Sdx/Pyr	
1974-Shute/Valera MES	Macro		19/46 (41.3%R)				Central Luzon, Cavite, Mindoro, Palawan, Rizal
1975-Shute/Valera MES	Macro		46/166(27.7%R)				Abra, Isabela, Quirino, Cagayan, K. Apayao, Central Luzon, Cavite, Mindoro, Palawan, Sulu, Tawi-Tawi, Basilan, N. Cotabato
1976-Shute/Valera MES	Macro		68/112(60.7%R)				Cagayan, K. Apayao, Zambales, N. Ecija, Rizal, Occ. Mindoro, Palawan, Sulu
1977-Shute/Valera MES	Macro		78/113(69.0%R)				Isabela, Quirino, K. Apayao, N. Vizcaya, Rizal, Occidental Mindoro, Palawan, Sulu
1978-MCS	Macro		61/104(58.7%R)				Isabela, Quirino, Cagayan, N. Vizcaya, Bulacan, Bataan, Zambales, Rizal, Occ. Mindoro, Aurora, Palawan, Quezon, Sulu, Tawi-Tawi, Davao
1979-MCS	Macro		55/90(61.1%R)				Isabela, Cagayan, Ifugao, Q.C. Rizal, Cavite, Aurora, Palawan, Quezon, Sulu, S. Cotabato
1980-MCS	Macro		21/35(60.0%R)				Isabela, Cagayan, Bulacan, Bataan, Caloocan City, Rizal, Or. Mindoro, Palawan, Sulu
1981-MCS	Macro		41/46(89.1%R)	0/25(100%S)			Cagayan, Bataan, Rizal, Laguna, Palawan, Quezon, Or. Mindoro, Sulu, Basilan
1982-MCS	Macro		22/38(57.9%R)	0/38(100%S)			Rizal, Camarines, Norte, Agusan Norte, Davao Norte
Sarkovski *et al.* (NAMRU)	Macro		9/14(64.3%R)				
	Micro	9/9(100%R)		9/42(21%R)	2/9(22.2%R)		Occ. Mindoro
1983-MCS	Micro		18/31(58.1%R)	1/31(3.2%R)			Bulacan, Cagayan, Quezon, Eastern Samar, Agusan Norte
1984-MCS	Micro		5/13(38.5%R)	0/4(100%S)			Rizal, Occ. Mindoro
1985-MCS	Micro	4/4(100%R)	6/11(54.5%R)	0/8(100%S)			Bulacan, Rizal
Sarkovski	Micro	3/59(5%R)		4/59(6.8%R)			San Lazaro Hospital
1986-MCS	Micro	10/12(83.3%R)	10/12(83.3%R)	0/5(100%S)	0/5(100%S)		Bulacan, N. Ecija, Rizal, Bataan, Quezon
Watt, *et al.*	Micro	4/5(80%R)					San Lazaro Hospital
1987-MCS	Micro	17/26(65.3%R)	12/21(57.1%R)	0/3(100%S)	0/18(100%S)	2/7(28.6%R)	Quirino, Bulacan, Bataan, N. Ecija, Quezon, Negros Oriental, Zamboanga
Long *et al.*	Micro	27/144(18.7%R)					
Sanofi (MCS/RITM)	Micro	8/8(100%R)		0/8(100%S)	0/8(100%S)		RITM Laboratory
1988-MCS	Micro	12/22(54.5%R)	0/20(100%S)	0/15(100%S)	0/22(100%S)	6/19(31.6%R)	Mindoro, Aurora, Quezon, Laguna, Bulacan
1989-MCS	Micro	4/6(66.6%R)	0/6(100%S)	0/2(100%S)	0/6(100%S)	2/5(40%R)	Bulacan, Quezon, Laguna, Bataan, Or. Mindoro, Cagayan and Caloocan City
MCS/RITM (Halofantrine Clinical Trial)	Micro	14/15(93.3%R)	0/15(100%S)	0/28(100%S)	0/15(100%S)	0/12(100%S)	
1990-MCS	Micro	5/13(38.5%R)	0/12(100%S)	0/1(100%S)	0/12(100%S)	0/9(100%S)	K. Apayao, Bulacan, Palawan

slopping stream banks, herbage cover and larvivorous fish (Ejercito, 1936). *Gambusia affinis* was introduced in Manila by the Americans from Texas. Since that time onwards scientific studies of the epidemiology of malaria, vector bionomics and various control measures were undertaken. These became the basis for launching a national malaria control program in 1953.

From 1952-1954 a DDT Pilot Project in Mindoro was conducted. After demonstrating that DDT is an effective and economical method of controlling malaria, WHO and the US government fully backed the Philippines for a nationwide DDT spraying operation. The result was quite successful that by 1956, the control objective was changed to an eradication program. In 1959, malaria was dramatically reduced to about 1.2/1,000 population that everyone was hopeful that malaria can be eradicated.

It was unfortunate for the program that in 1959, the government started its reorganization. The Department of Health was required to decentralize the Malaria Eradication Service (MES) and placed it under the direct administrative control of the eight Regional Health Offices. MES was reorganized and performed staff functions. The next six years of the program saw resumption and resurgence of malaria. The objective of malaria eradication became dim. After a review of the program and an administrative campaign to centralize it once more, Republic Act 4826 was passed in 1966. This act mandated a centralized nationwide eradication program under one director with the assistance of USAID and WHO. Twenty American Peace Corp Volunteers were also assigned with the Program. Annual Parasite Incidence (API) was maintained at around 3/1,000 population. With the phasing out of USAID and WHO beginning 1973 and the diminishing logistics support of the government, API went up to around 6/1,000 and maintained at that level until MES was again decentralized and integrated to the Field Health Services through Executive Order No 851 in 1983. The problem of inadequate resources remained, vector control was limited to containing flare-ups and house spraying was selective and partial. Only bedrooms were sprayed. Malaria situation worsen and API steadily rose to about 7.8/1,000 by the end of 1986. In 1987 API dramatically went up to 14.5/1,000 population (Table 3, Fig. 3).

2. Current status of the program:

After the famous bloodless people power revolution of 1986, major changes in the organization and management were introduced by the new DOH leaders. Among the programs evaluated was the malaria control program. Executive order No. 119 dated January 30, 1987 was issued giving opportunities for the new management to reorganize and introduce innovative approaches to malaria control. Noting the deteriorating endemicity level of malaria and considering the lessons experienced from the previous decentralization, the new organization was restructured to be semi-vertical wherein a strong vector control service was placed directly under the direction of the Provincial Staff while case finding and treatment was integrated at the District Health levels.

The present MC program is strengthened by a five

Table 3 Parasitological Indices from 1957-1991

Year	Population at risk	SPR	API	ABER	Remarks
1957			24.20		
1958			2.80		
1959			1.21		
1960			5.53		Decentralization
1961			4.53		
1962			6.91		
1963			3.43		
1964			4.44		
1965			6.65		
1966			1.86		Centralization
1967			3.29		(RA 4826)
1968			2.23		
1969			1.90		
1970			3.22		
1971	11,433,109	3.04	3.24	10.70	
1972	11,575,121	3.30	2.40	7.10	Martial law
1973	11,857,517	7.20	6.10	8.40	(1972-1981)
1974	11,157,908	7.33	7.47	10.20	
1975	12,028,879	6.40	5.80	9.10	
1976	13,914,846	5.40	5.20	9.40	
1977	14,298,709	7.80	6.00	7.70	
1978	14,441,556	10.40	7.30	6.90	
1979	14,631,273	8.60	5.90	6.80	
1980	15,813,846	11.60	6.60	6.00	
1981	14,000,000	12.30	5.80	4.80	
1982	16,297,226	10.70	5.90	5.50	
1983	15,142,521	12.50	5.90	4.80	integration (EO 851)
1984	15,483,321	15.10	6.90	4.60	
1985	11,111,366	23.50	9.20	3.90	
1986	13,128,205	16.50	7.80	4.70	People power revol
1987	10,587,598	16.70	14.50	10.50	MCS strengthened
1988	11,126,133	11.40	13.90	12.01	(EO 119)
1989	11,666,219	7.50	9.90	12.70	
1990	11,348,235	7.30	7.30	10.00	WB assistance
1991	11,871,273	6.81	7.28	10.68	

From Anmual Reports, MCS: 1957-1991

year loan from the World Bank which took effect July, 1990.

2.1 Objective

The general objective is the reduction of malaria incidence to 1/1,000 population by year 2000.

2.2 Policies and strategies

The program adopted the tactical variants advocated by the World Health Assembly in 1978 in the context of PHC, namely: reduction of malaria mortality and morbidity and reduction of malaria prevalence and incidence. It is now moving to the initial implementation of the 4th tactical variant.

The key strategy is to implement effective vector control measures in areas with API>5/1,000 population complemented by the rational use of appropriate anti-malaria drugs. In areas with API<5/1,000, intensified case finding and immediate treatment through PHC supplemented by an active health information system directed towards personal protection and prevention from malaria. In some selected areas, when appropriate, biological-environmental management measures are implemented through community participation.

a) Policies

(1) Standard organization at regional and provincial levels;

(2) Setting aside funds for hiring spraymen and canvassers and paying their travel allowances by respective Regional/Provincial Health Offices;

(3) Issuance of guidelines on planning and field operations;

(4) Creation of malaria coordinators at regional, provincial, district and municipal health levels;

(5) Securing funds from different funding sources such as General Appropriation Act, World Bank, Area Development Projects, WHO, USAID, NGO, etc.;

(6) Staff development, training, transfer of technology;

(7) Operational research in collaboration with other research institutions and continued testing of insecticides and anti-malaria drugs;

(8) Continued monitoring of parasite resistant strain;

(9) Fielding central office monitoring and evaluation teams;

(10) Centralized procurement of insecticides, drugs, equipment and logistical support to field services;

(11) Linkages to NGO, GO and international agencies;

(12) Production of test kits and incubator for *in vitro* research in coordination with WHO/CIDA for world distribution.

b) Strategies;

(1) Stratification of endemic areas and application of appropriate control measures per stratified area (Fig. 1)

(2) Emphasis on vector control in areas with API>5/1,000

(3) Intensified/stratified case finding and immediate treatment and parallel development of voluntary post involving community participation in blood collection and treatment;

(4) Rational use of anti-malaria drugs considering resistant strain;

(5) The use of the different types of insecticides shall be staggered by area and by interval to prevent the early development of insecticide resistance of mosquito vector(s);

(6) Supplementary use of impregnated mosquito net as a control measure;

Stratification API/1,000	Malaria Control Measures										
	Surveillance						Vector control			Personal Protection	IEC
	Mod. Vig.	ACD/ PCD	Blood exam.	Epid. invest.	Treat ment	Reme- dial	Chemi- cal	Biolo- gical	Environ- mental		
Malaria freed	<1/1,000	—	—	✓	✓	✓	—	—	—	—	✓
<5/1,000 API		✓	✓	✓	✓	✓	Focal	Selec- tive	Selec- tive	✓	✓
5-10/1,000 API		<7/1,000	✓	✓ <7/1,000	✓	—	1 cycle spray	✓	✓	✓	✓
>10/1,000 API		Optional (PCD only)	Optio- nal	—	✓ (Clinical only)	—	2 cycle spray	✓	✓	✓	✓

Figure 1 Malaria control strategie in different stratified areas

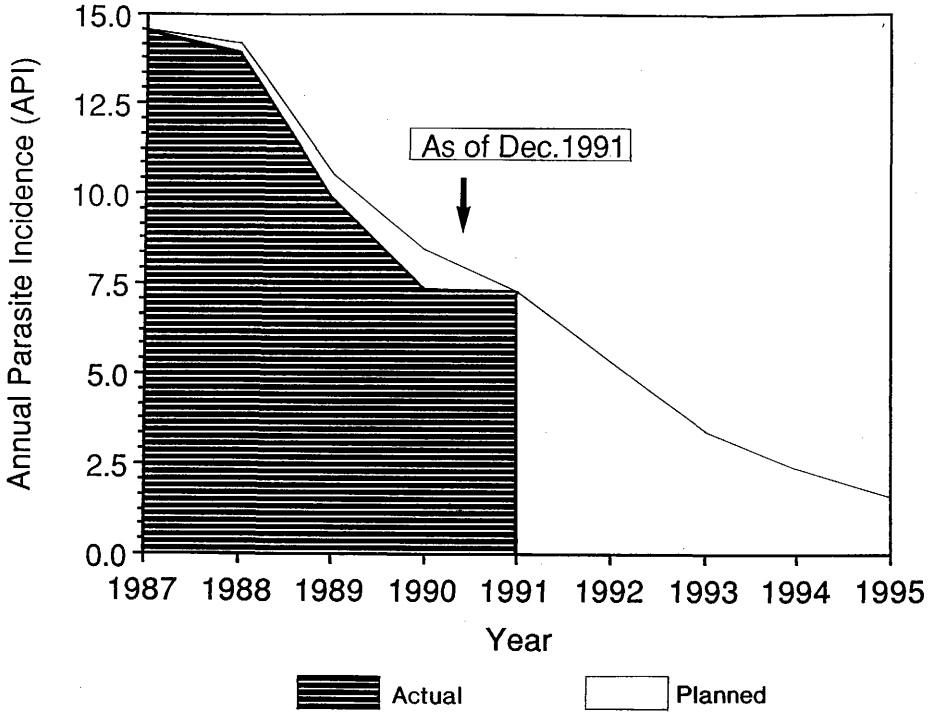

Figure 2 Planned and actual API reduction Malaria Control Service, 1987-1995.

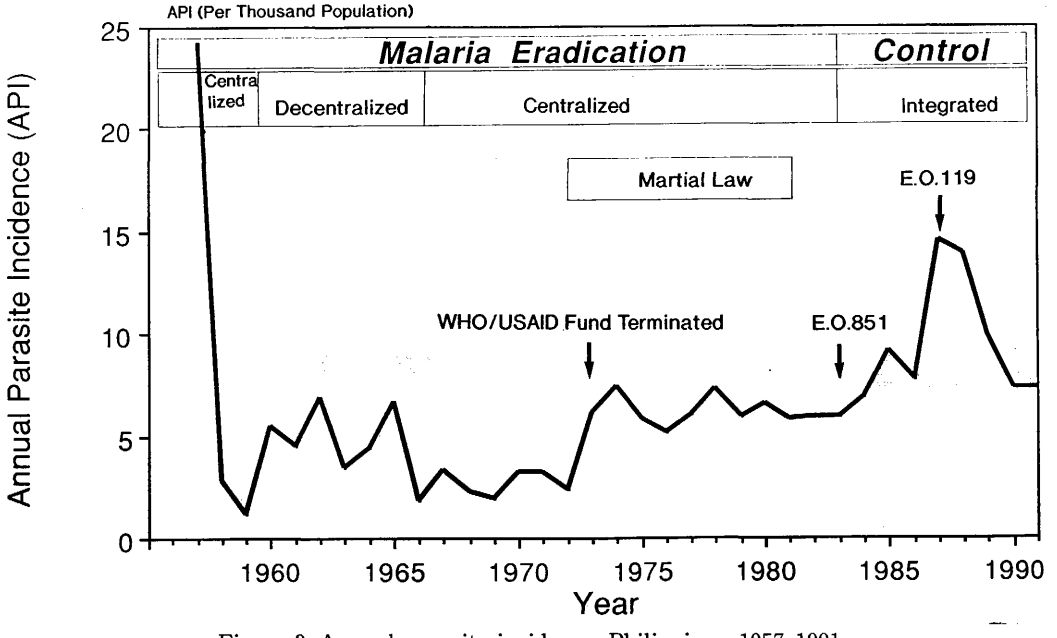

Figure 3 Annual parasite incidence, Philippines, 1957-1991.

(7) Establishment of integrated microscopy center at district level: Clinical diagnosis is advocated in highly endemic areas and where appropriate;
(8) Intensification of IEC/health informations;
(9) Scheduled monitoring and evaluation

3. Accomplishments

The national Annual Parasite Incidence (API) has been reduced from 14.5/1,000 in 1987 to 7.3/1,000 population ending 1991. There are twenty-one provinces which have attained an API less than 1/1,000 population and

three non-endemic out of 75 provinces as of 1991. It is projected that by 1994, the average API will be around 2.5/1,000 population (Fig. 2).

3.1 Comparative accomplishments: 1987-1991

Particular	1987	1988	1989	1990	1991
1. API	14.5	13.9	9.9	7.3	7.28
2. SPR	10.5	11.4	7.8	7.3	6.81
3. ABER	13.8	12.1	12.7	10.0	10.68
4. Smear exam.	1,113,784	1,356,024	1,478,968	1,183,370	1,268,284
5. Positives	154,091	154,943	115,542	86,172	86,392
-*P. falciparum*	104,281	107,373	76,730	52,781	56,867
-*P. vivax*	48,285	46,779	38,079	32,983	28,778
-Mix infections	1,525	791	733	408	747
6. Houses sprayed	270,729	651,041	686,539	671,624	662,004

4. Problems/Constraints

4.1 Administrative

(a) Untimely/delayed release of funds; funds still inadequate;

(b) Lack of trained malaria personnel. No takers, salary being not commensurate with difficult tasks;

(c) Malaria is presently a low priority program by RHO/PHO. Malaria is now the 10th leading cause of morbidity and no longer among 1st 20 cause of mortality.

4.2 Technical

(a) Parasite resistance to some anti-malaria drugs

4.3 Operational

(a) Refusals to house spraying with DDT;

(b) Population movement;

(c) Lack of transport facilities;

(d) Incomplete treatment of cases due to attitude of patients;

(e) Apathy of community in participating in the control program;

(f) Uncertain peace and order condition in many highly endemic areas.

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NEUROPATHOLOGICAL OBSERVATION OF FUNGUS DISEASES IN THE TROPICS

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Among about 100,000 species of fungus, it is said that pathogenic fungi are about 100 species.

In deep-seated fungus diseases, there are primary mycosis and secondary mycosis. Secondary mycosis is very common throughout the world as an opportunistic infection. However, as for primary mycosis, fewer cases are seen and mostly endemic. It is particulary significant in the tropics, but very rare in Japan except chromomycosis and a few cases of cryptococcosis.

MATERIALS AND METHODS

Cases with cerebral lesions were used among materials hitherto gathered. Most of cases of tropical mycoses were from institutions of foreign countries.

PATHOLOGICAL CHANGE OF OPPORTUNISTIC FUNGUS DISEASE

As an opportunistic infection, candidiasis is spreading hematogenously with many diseases including SLE as primary disease. There are numerous spotty hemorrhage and lesions are also present in the ventricle. In the case like this, fungal elements of Candida stretch, morphologically simulating to true filamentous fungi, with tissue reaction being very rare.

In aspergillosis, lesions resemble to those by Candida, but hemorrhagic focus of Aspergillus is rather larger. Histologically, embolic lesions are present.

In cryptococcosis, granulomatous primary focus is sometimes found in lung, and when the host falls into the immunosuppresive condition, systemic cryptococcosis becomes present through hematogenous spread. It is evident that there are many fundamental diseases which bring about the decreasing of cellular immunity. It is also characteristic that multiple cystic lesions are developed (Fig. 1). Histologically, it is meningoencephlitic lesion without tissue response (Fig. 2).

In mucormycosis, hemorrhagic infarction is often observed, whereas granuloma is rare.

Nocardiosis is mainly caused by *Nocardia steroides* which produces abscesses in lung and brain. The disease currently occurs on the immunocompromized host after organ transplantation. Delicate filaments are present (Fig. 3).

As a typical primary fungus disease, histoplamosis is endemic in some regions of USA along the Ohio and Mississippi River, but cases have also been reported from Central and South America, Africa and very rarely from Indonesia. Clinical signs resemble closely to

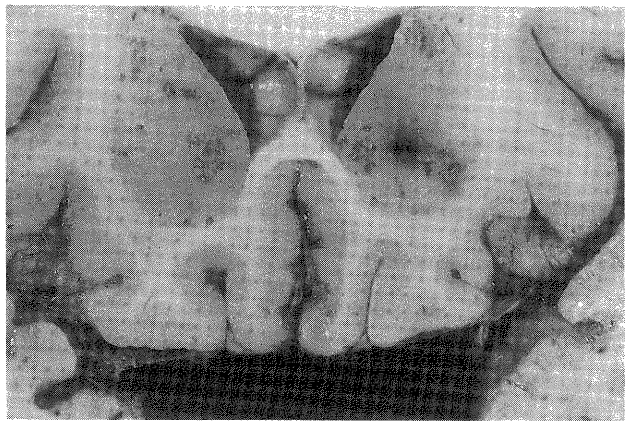

Figure 1

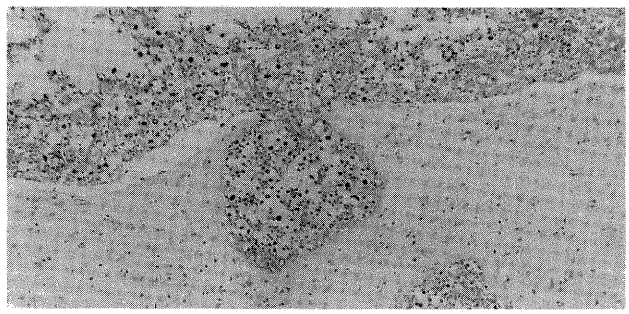

Figure 2

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Presented at XIth International Congress of Neuropathology, Kyoto, September, 1990.
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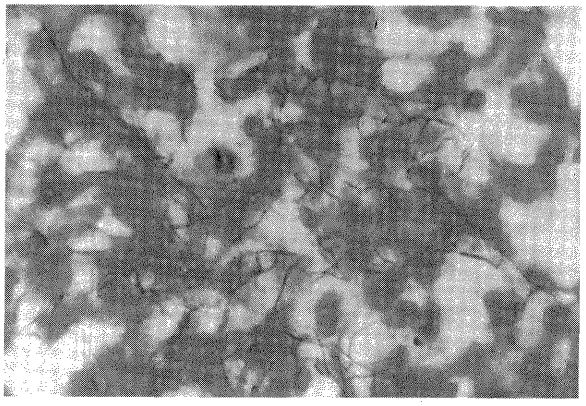

Figure 3

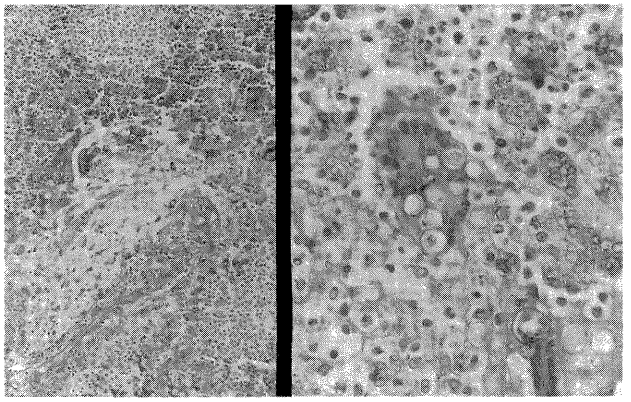

Figure 4

tuberculosis. Involvement of the brain in this disease is uncommon, however when involved, there appeares meningitis type with meningoencephalitic feature or abscess formation including yeast cells. Although very rare in case, epithelioid cell granuloma with caseous necrosis closely mimic to tuberculosis is developed. Fungal elements of *Histoplasma capusulatum* are seen in the central zone of caseous necrosis, which were stained using anti-*Histoplasma capusulatum* antibody made by ourselves.

General Pathological Aspect in Tropics

African histoplasmosis and Lobo's disease are less common and no cases with lesions of Central Nervous System (CNS) have been reported.

North American blastomycosis is endemic in USA and Canada, but it has also been reported in Venezuela, Congo and South Africa. CNS is involved in the ratio of 3-20% and the lesion presents as the combination of suppurative and granulomatous reaction. Meninges, basal ganglia, cerebellum and spinal cord are known to be involved.

South American blastomycosis, synonym for paracoccidioidomycosis, is mainly observed in Latin American countries. Lesions are mainly seen in lung, lymph node, adrenals and liver. Incidence of lesions in the CNS is about 10%. Cerebral involvement presents as granulomatous lesion and there are two forms; meningitis and granuloma (Fig. 4). Yeasts 10-60 μm in diameter with multiple buds are seen in the granulomatous lesion. In meningitic forms, the lesion tends to be mainly localized on the base of the brain. Cells positive to GFAP proliferate around the abscess. Around fungus agent positive to PAS reaction, there appeared numerous macrophages stained by anti-Human alveolar macrophage (HAM).

Coccidioidomycosis is typical primary mycosis due to the fungus, *Coccidioides immitis*, which has the most infectious pathogenesis. The disease has a distribution in Western parts of USA, Mexico and the Pacific side from the Andes of South America, but more particularly in San Joaquim Valley of California and Arizona of USA. Lung, spleen and brain are frequently involved. Dr. Hungtington reported that the cerebral lesions were seen in 82 cases among 142 autopsy cases (58%). In the CNS, the disease can present as a meningitis and meningoencephalitis (Fig. 5), and the formation of granulomas is often observed. Fungi occur in tissue as sac fungi containing endospores. Meningeal and submeningeal glias are proliferated, and within the inflammatory change of the meninges, macrophages by HAM are increasing. In granulomatous type of coccidioidomycosis, glias proliferate around the granuloma and a lot of macrophages are seen in granuloma by GFAP•PAS and HAM.

As to rhinosporidiosis, two cases have been report-

Figure 5

ed with the involvement of spinal cord, however, no cases with the lesion in the CNS.

Cladosporiosis in the CNS is a general term for the diseases caused by brown fungi, so-called Dematiacea such as genus of *Cladosporium, Phialophora, Foncecaea, Exophiala*. However, the name is usually restricted to those only by *Cladosporium* spp. such as *C. trichoides, C. bantiarum, C. gougeroti, C. carionii*. When using the restrictive name, the diseases by the other brown fungi are called to be chromoblastomycosis or chromomycosis. Many cases have been reported in Europe, North and South America, Africa, Indonesia, Taiwan, Japan, etc.

In tropics, there are many cases of diseases by brown fungi. However, in Japan, there are a few cases by *Cladosporium* spp., while as to chromomycosis, about 20 autopsy cases are reported, most of wihch are of children under 15 years old. It is also characteristic that cerebral involvement is present in many cases (Fig. 6).

In chromomycosis, it is characteristic that brownish black lesions are present. Generally, granulomatous lesions are developed, and the meningitic lesions are often seen with the involvement in Virchow-Robin's space. Multinucleated giant cells are phagocyting fungus with the proliferation of glias around. Macrophages are also increased. In only one case, within the severe inflammatory cell infiltration, numerous B-cells were observed, but no T-cells.

The formation of multiple granulomas are thought to be by means of hematogenous spread. However, in human cases, as the formation of lesions and their early stage cannot be observed, we carried out the experiment using mice.

Mice were intraveneously injected with 2 strains of *Exophiala dermatitidis* demonstrating different virulence which were isolated from human brain and lymph node.

Figure 6

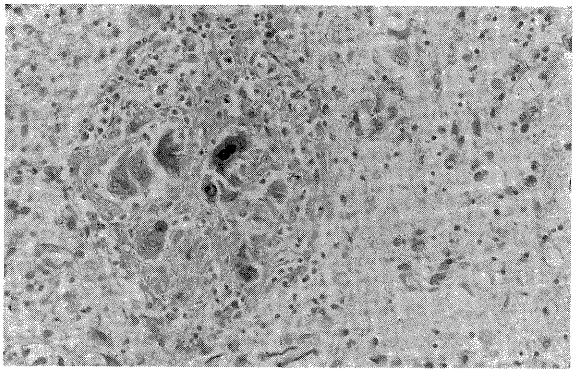

Figure 7

Observing periodic change of the lesions, we studied the course to produce granuloma (Fig. 7).

Lesions may occur in any part of the brain. In some cases, meningitis was developed, but in most cases, abscess with fungal elements was produced.

On the 7th day after injection, prominent glioses were present. On the 17th day, appearance of macrophages became marked, and there was a tendency to produce granulomas.

SUMMARY

The cerebral lesions of fungus diseases are of three types; meningitis type, encephalitis type and mixed type of the both. As to inflammatory changes, the translation from suppurative inflammation to granuloma was observed.

Secondary mycosis arises in immunocompromized host by prominent proliferation of fungi following bacterial bashing induced by antibiotics. It is usual that there is no tissue response.

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ALTERATIONS OF SPINAL CORD IN JAPANESE B ENCEPHALITIS

MASAO KISHIKAWA

Abstract: The cytopathologic changes of Japanese B encephalitis (JBE) are basically similar to those of other forms of arbovirus encephalitis. Because the entire central nervous system including the spinal cord is involved to varying degrees, the nomenclature of JBE should actually be Japanese B panencephalomyelitis.

INTRODUCTION

Japanese B encephalitis (JBE) is a tropical neurological disease found in the countries of east and south Asia, such as Japan, Korea, China, Taiwan, Malaysia, Indonesia, Thailand, Nepal and India. Since the isolation of the pathogenic virus by a Japanese neuropsychiatrist in 1934, the disease has been called Japanese B encephalitis (Hayashi, 1937). As a result of the geographical distribution, very few neuropathological details of JBE can be found in European and American textbooks. Descriptions of the spinal cord lesions are also rare in the textbooks.

MATERIALS AND METHODS

Spinal cord and brain specimens of nine JBE cases were available from two children, two middle-aged and five aged patients who underwent autopsies (Table 1). The duration to death was 4 days to 307 days. All of these JBE cases were confirmed serologically. Neuropathological investigations were performed histopathologically and immunohistopathologically.

RESULTS

The surfaces of the brains usually showed congestive and sometimes edematous changes. But no suppurative alterations were observed. The thalamus, corpus striatum and substantia nigra were always affected prominently. Hemorrhagic lesions were frequently found in the thalamus. Petechiae and small foci of necrosis were also detected not only in the thalamus but also in other parts of the cortex and centrum semiovale. Histologically, focal and small necroses of the cerebral cortex were frequently observed. Vacuolar changes of

Table 1 The cases of spinal cord studies on Japanese B encephalitis

No.	Age	Sex	Antibody	Duration	Brain	Others
1	4 mos.	M	↑ ↑	4 days	935 g	Brain edema
2	5 yrs.	M	↑	8	1,300	Brain edema
3	47	M	↑ ↑	18	1,370	
4	51	F	↑ ↑	307	—	Only spinal cord
5	65	M	↑ ↑	14	1,250	
6	65	F	↑ ↑	68	1,140	Cervical cord
7	69	M	↑ ↑	4	1,300	
8	72	M	↑ ↑	18	1,360	Brain edema
9	82	M	↑	12	1,100	

Duration: course from onset to death (days)

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the cerebral cortex and white matter were also occasionally found. Glial nodules were one of the most conspicuous alterations. The reactive cells were astrocytes and microglias. Neutrophils were also mixed in the glial nodules in the cases lasting only 4 days. Perivascular lymphoid cell cuffing was easily detected. Relatively well circumscribed demyelinated lesions were also commonly observed. The axons of these foci were almost completely preserved. Cases of long duration, however, showed a few axonal swellings. Immunohistochemical staining for anti-JBE virus antibody (courtesy of Dr. Sata, JNIH: Tokyo) showed positive findings in the neurons of the thalamus (Fig. 1a). The substantia nigra was another one of the most severely affected sites. Marked gliosis and neuronal degeneration with dropping out of neuromelanin were easily observed. Although the entire cortex was involved mildly to severely, the thalamus, midbrain and corpus striatum were mainly and severely affected in all cases. The cerebellum, pons and medulla oblongata were also involved to varying degrees.

With regard to the spinal cord, macroscopically, moderate to marked congestion was found in almost all JBE spinal cords. Microscopically, the spinal lesions of JBE displayed basically the same appearance as those in the brains. Perivascular lymphoid cell cuffing, glial reactions such as glial nodules and neuronophagia (Fig. 1b), petechiae and myelin loss were detected. Of all the spinal levels, the lumbar portion was affected most severely. Neuronal loss of the anterior horn and rarefaction were detected occasionally. Perivascular lymphoid cell cuffing was clearly evident, while thickening of the vascular wall was not observed in JBE. Central chromatolysis was found frequently in the motor neurons of the anterior horn. Three of the nine cases showed some degree of alterations in the lateral pyramidal tract, but with indistinct circumsription. Two cases showed mild demyelination and/or myelin pallor (Fig. 2a), and the other case showed moderate vacuolar changes in the pyramidal tract. The pyramidal lesion was more prominent at the thoracic level. The patient who died after only four hospital days also showed vacuolar changes in lateral pyramidal tract. A few cases showed intracytoplasmic vacuolar changes in the large neurons of the anterior horn. There was no confirmation of the existence of neutral fat in these vacuolar lesions. Perivascular lymphoid cell cuffing and chronic inflammatory cell infiltration were also observed in both the motor and sensory nerves of the cauda equina. Demyelinating foci were occasionally detected in the peripheral nerves (Fig. 2b).

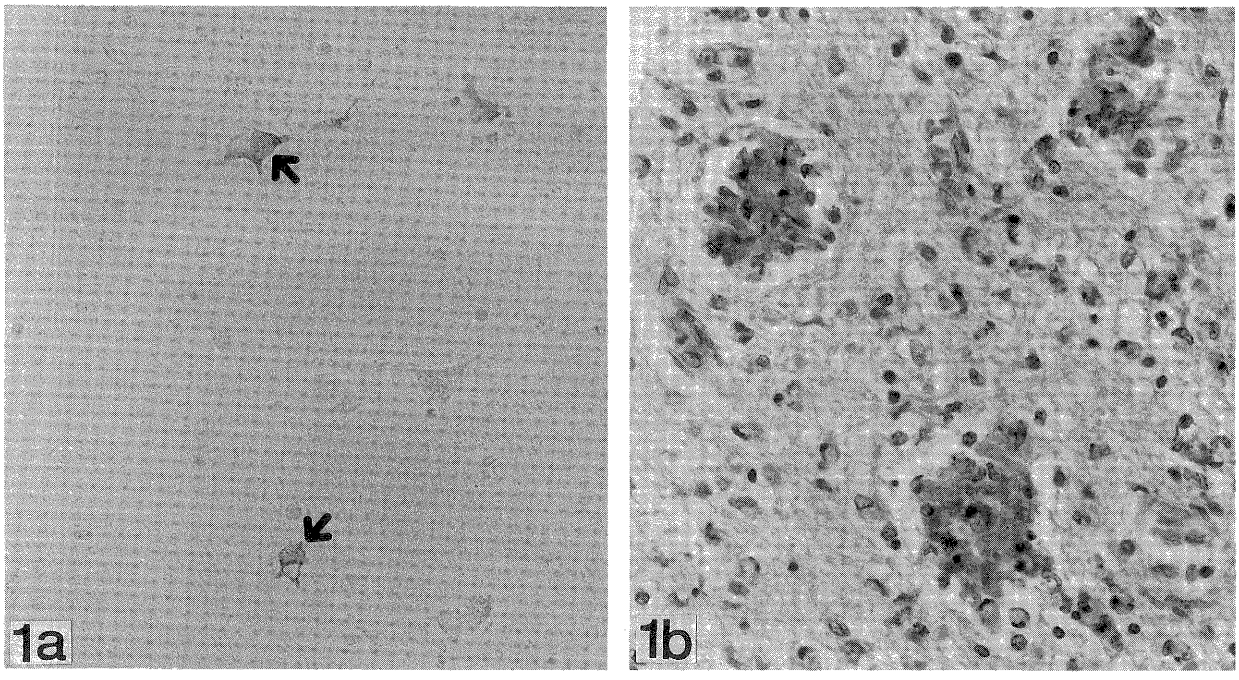

Figure 1 a. Immunohistochemical staining for anti-JBE virus antibody. Neurons positive for anti-JBE virus are detected (arrows). Thalamus of case No. 3. ABC method, ×300. b. Neuronophagia of the spinal motor neurons. Marked glial reactions are observed in the spinal anterior horn of case No. 1. HE stain, ×300.

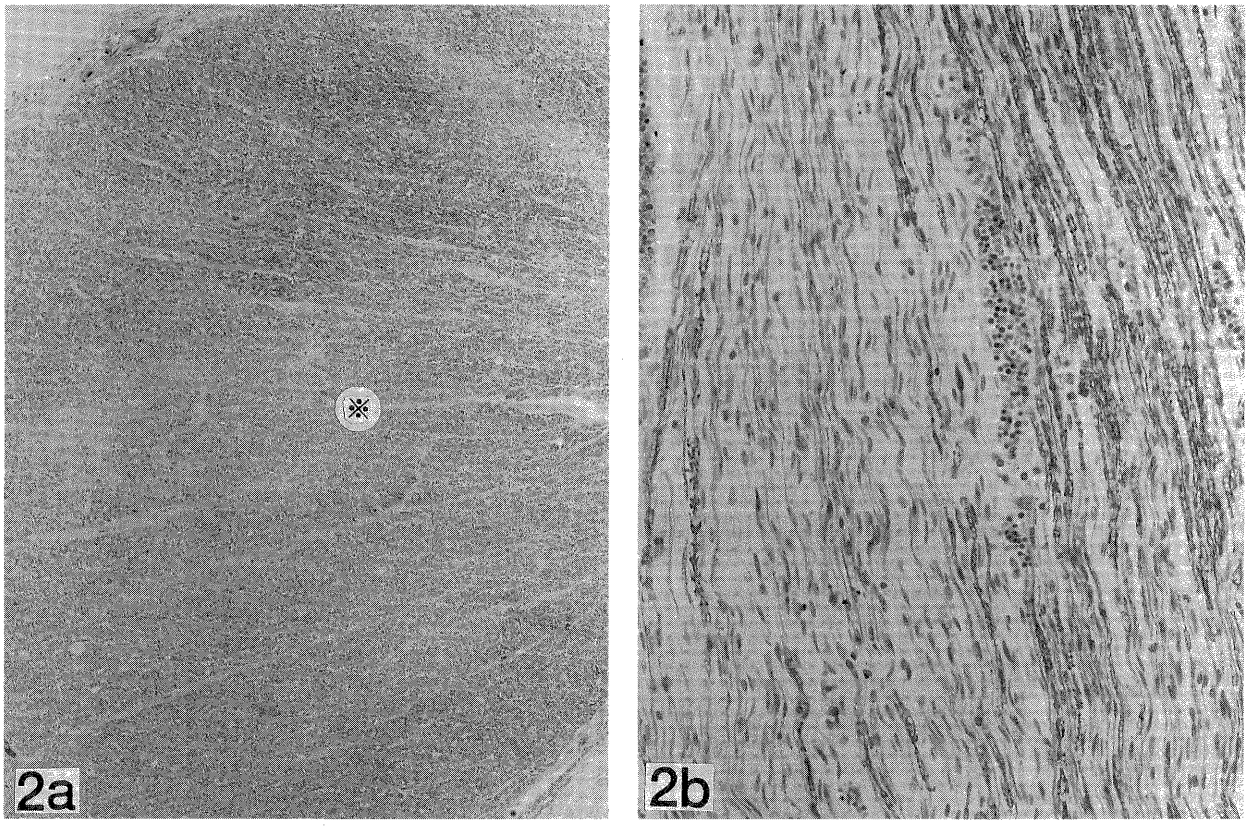

Figure 2 a. Alteration of the lateral pyramidal tract. Mild demyelination and/or myelin pallor is seen at the lateral pyramidal tract(*) of case No. 7. KB stain, ×30. b. Sensory nerve of the cauda equina of case No. 5. Demyelinating lesion (left side) and preservation of myelin (right side) are mixed. KB stain, ×150.

DISCUSSION

As a result of the geographical distribution of the disease, very few neuropathological details of JBE can be found in European and American textbooks. Before discussing the neuropathological features, it will be useful to present a brief epidemiological introduction of JBE in Japan.

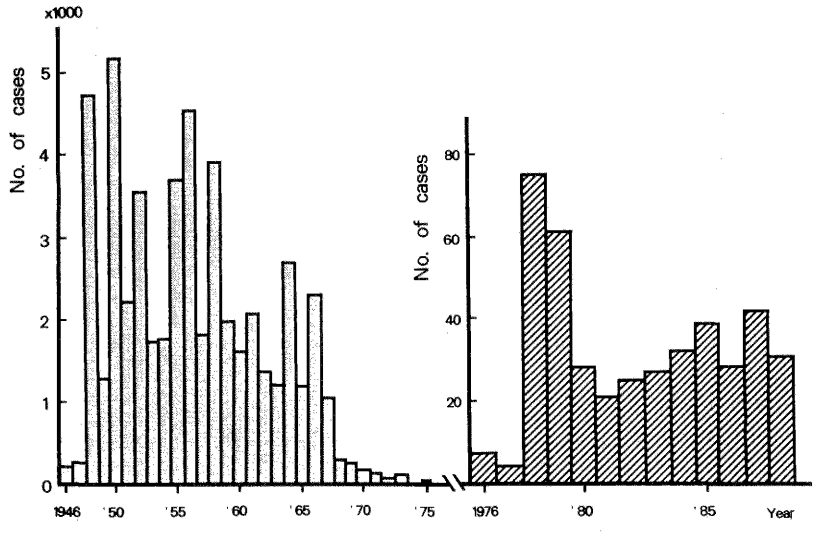

Figure 3 The number of cases of Japanese B encephalitis in Japan: 1948—1988.

JBE has been the subject of a nation-wide statistical surveillance in Japan since 1946 and has been designated as a legal epidemic disease since 1954. The number of cases peaked in 1950, with 5,196 patients and a morbidity of 6.2. From 1948 to 1967, more than one-thousand patients were detected each year. In recent years, however, a total of less than 50 patients (Fig. 3) and 0.0 morbidity are observed. JBE was seen predominantly in children and in aged groups. But in recent years, very few cases are detected among children because of vaccinations for JBE. The southwestern part of Japan, especially the island Kyushu, shows the highest incidence, while most of the northern island Hokkaido, located above a latitude of 41°N, has no JBE patients.

The morphological aspects of JBE are basically similar to those of other forms of arbovirus encephalitis, with the exception of Eastern equine encephalitis (Heffner, 1976). Macroscopically, brain edema and congestive changes are the most prominent features in the early phase. Although the inflammatory reactive cells are mainly neutrophils rather than lymphoid cells in Eastern equine encephalitis, the reactive cells of other forms of arbovirus encephalitis including JBE are mainly lymphocytes and glial cells (Heffner, 1976).

Very few details of the spinal changes can be found in the English literature. The spinal changes of JBE display basically the same features as those of other forms of arbovirus encephalitis, such as glial nodule, neuronophagia (Fig. 1b), petechiae and myelin loss. Perivascular lymphoid cell cuffing is also prominent, while thickening of the vascular wall such as that seen in HTLV-I associated myelopathy (HAM/TSP) (Akizuki *et al.*, 1989) is not observed in the spinal cord of JBE. Mild demyelination and/or myelin pallor (Fig. 2a) and moderate vacuolar alterations in the lateral pyramidal tract were detected in three cases in this spinal study. Some aspects of the alterations in the lateral pyramidal tract, which show indistinct circumscription of the lesion, might be secondary to proximal lesions such as those in the cerebral cortex and white matter. In the acute phase, however, edematous changes might play the role of vacuolar changes, because one case with lesions in the lateral pyramidal tract did not show lesions in the related cerebral areas and this patient died after only four hospital days.

Because the entire central nervous system including the spinal cord is involved to varying degrees, the nomenclature of JBE should actually be Japanese B panencephalomyelitis.

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Note

THE DEMOGRAPHIC ATTRIBUTES OF THE PROVIDERS AND UTILITIES OF THE SERVICES OF TRADITIONAL HEALING HOMES IN BENIN CITY, NIGERIA

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Received October 25 1991/Accepted September 1 1992

Abstract: In Nigeria, modern medicine exists side by side with traditional medicine. Despite the advantage of age enjoyed by traditional medicine, only allopathic medicine is officially recognised. A good number of the literature on the healing systems in Africa is repleted with derogatory comments about the person and work of the traditional healer (Oyebola, 1978; Ndeti, 1976; Saunders, 1953; Jahoda, 1962; Mbiti, 1976; Uyanga, 1979).

In spite of all these, WHO (1978) has continued to maintain that 80 per cent of the inhabitants of Third world countries seek health-help from the traditional healers and has consequently directed their integration into nations' health care delivery services.

All these heighten our curiosity about the real identity of this set of healers about whom so much have been said, but who have some-how managed to escape the scrutiny of analysts. The provision of information to fill this vacuum constituted the thrust of this work.

On the whole, the results in section A (Clients) showed that residents in Benin City employ the services of the tradomedical practitioners irrespective of social classes. In section B (Healers) we discovered that a good number of the myths concerning the traditional healers were unfounded. Furthermore, that for the most part, healers were not too different in social attributes from the average man in the population and are highly responsible people.

INTRODUCTION

WHO (1978) defined African medicine as: . . . the sum total of practices, measures, ingredients and procedures of all kinds whether natural or not which from time immemorial had enabled the African to guard against disease, to alleviate his sufferings and to cure himself.

This definition is quite embrasive and adequately captivates the world view of Africans.

Ndeti (1976), has observed that the knowledge of medicine is known in all human societies. According to him, the mere fact that a people has domiciled in a particular environment for a prolonged period of time is enough indication that they have to a certain extent acquired enough homeopathic adaptation to enable them survive.

African medicine is akin to the one Ndeti described above. Modern medinine on the other hand was introduced during the colonial rule and today exists contemporaneously with traditional medical system in most African countries. It is scientifically based and practised along the Euro-american model. Modern medicine is the dominant form of medical practice in most African nations. Dominance here is synonymous with government recognition. However, exidences abound which suggest a general insufficiency and inability of modern medicine to successfully cater for the health needs of Africans (WHO, 1978; Good *et al.*, 1979; Otayula, 1973; Ademuwagun, 1969).

Consequently, 80 per cent of Africans rely on the traditional healers to meet their health needs (Oyebola, 1981). Part of the explanation for this high utilization of traditional healing method also lies in the orientation of modern medicine which sharply contradicts traditional orientation. Modern medicine is founded on science and accordingly limits it's explanation to what it can empirically verify. But while it is true to say that the reflection

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of scientific ethics in allopathic medicine is in consonance with the world view of western societies, the same cannot be said of African societies, whose views on the phenomenon of disease causation incorporate the natural and the unnatural. This is evidenced by the dual health-shopping commonly found in Africa (Asuni, 1979).

The general attitude of the various governments in Africa, to the traditional healers has been one of apathy. Such has led to the official recognition of modern practitioners only, despite numerous criticisms of orthodox medicine and high patronage enjoyed by traditional healers.

Writing on this despicable attitudes to the traditional healers, Ndeti (1976) observed that he is often referred to as being the 'Jack of all trades but a master of none' by his professional colleagues in modern medicine. Like a philosopher, he is said to know everything about all diseases safe a particular one. Continuing, he noted that they claimed that his diagnoses and therapies are married by an aura of mystery. While others concluded that because he lacks the necessary scientific training in medical sciences, he does not appreciate the magnitude of such things as germs theory and so much of his works continue to be a mere guesswork. While those who conceed that he gives herbal prescription also maintain that they are for the most part unscientific.

Yet others see him as a clever rascal, an extortionist and a barrier to the wheel of progress in the rural area, while some others continue to refer to them as witch-doctors. Writers like Mbiti (1976), Torrey (1973), Sempebwa (1983) have devoted part of their works to looking at this attitude to the works and person of the traditional healer.

The net result has been a continued mystification of the work and person of the traditional healer. While such mythologies and mystifications linger, WHO (1978), has called for the integration of traditional healing methods into the mainstream of nations' health-care delivery services.

All these exacerbate our curiousity and further inflame the nagging question of who a traditional healer is and what sort of people utilize his services. The provision of information to fill this gap, is the prime motivator of this work. It is hoped that this will stimulate others to further research in this field and create a basis for comparison.

METHODOLOGY

The study involves 100 respondents. Of this figure, 50 respondents were drawn from the class of traditional healers, while 50 came from respondents who have at one time or the other, sought and obtained the assistance of traditional healers. The purpose of adding the latter group is to enable us cross-check some of the responses of the traditional healers as well as illuminate the issue of the kind of persons that constitute his clients.

The purpossive method of sampling the universe was employed. Furthermore, the data were generated through the use of a questionnaire and indepth interview. The questionnaire was divided into 2 sections: A and B. Setion B was exclusively meant for the traditional healers and was designed to elicit information concerning the demographic attributes of healers, the mode of training, the practice and efficacy of healers drugs. However, in this present work only the demographic attributes was taken into cognisance. Section A was mainly devoted to bringing forth information relating to the social characteristics of the clients of healers.

It suffices to say that the questionnaire took a semi-structured form. The questions were open and close ended. The essence is to allow for a wide range of responses especially in areas where the subject matter does not lend itself to easy grasping. All interviews were conducted and recorded by the researcher.

RESULTS

Table 1 Going to the traditional practioner by age and sex

Ages	Male	Female	Total
1—15	0	0	0
16—30	7	10	17
31—45	8	7	15
46—60	11	4	15
61—75	2	0	2
61+	0	1	1
Total	28	22	50

Section A

The data collected under this variable revealed that 28 males as against 22 females were sampled. It is the opinion of the researcher that it will be premature to conclude that more males than females utilize the services of the traditional healers in Benin City.

The reason for this is that since the sampling technique employed was geared towards interviewing only those who have received the services of healers, it may well be that what our samples simply show is that more males than females were willing to report and were

consequently sampled. This however does not completely remove the oddity in this results, since in the society under study, women are generally saddled with the care of young children.

Age: We can further analyse Table 1 by looking at the ages of respondents. In order to examine this variable, the ages of respondents were calibrated as shown above.

As Table 1 shows, nobody was interviewed in the age limit 1-15 years. We like to believe that this finding is quite apt. It would have been foolhardy considering the type of dreadful stories that abound concerning the traditional practitioner for a 15 year old boy to seek the services of one alone (Ndeti, 1976; Torrey, 1972; Unschuld, 1976 ; Oyebola, 1981). Thirty-four per cent of the respondents fell into the age category 16-30 years. Another 30 per cent each fell within age limits 31-45 and 46-60 years respectively. By the same token, 4 per cent fell into age category 61-75 and 2 per cent in cohort 76 and above. It would seem from this distribution that in Benin City, the bulk of the people who seek the services of the traditional healers come from age cohort 16-60 years. It is observable that no significant pattern or frequency of utilization exists among age limits 16-30 years, 31-45 years and 46-60 years. Similarly, the findings of only 4 per cent and 2 per cent recorded in age categories 61-75 and 76 and above do not look too strange. It is expected that by the time a man attains those age brackets, his children would be matured enough to "cater" for themselves.

We can account for the lack of any significant differences recorded in age category 16-60 years on the ground that this group contains those who are either preparing or just given out in marriage and are anxious about many "good things" of life and the fact that it also encompasses parents who for diversified reasons are either looking after themselves, their children or household in general.

Education: This is another area where the views of respondents were sampled. Subjects were asked to report on the highest educational qualification attained. To facilitate this, education was divided into 3 phases: the literate category which comprised of everybody who had post secondary qualification of any kind, the semi-literate group which include everyone who read beyond post primary but not above secondary form 5 and the illiterate cohort comprising all those who had no formal education of any kind, or read below primary 6. Their responses were organised and further regrouped in terms of sex. The results showed that under the literate grade, 6 male respondents as against 4 females read beyond secondary form 5. Going further, we discovered that 14 males as opposed to 12 females fell into the semi-literate cohort. Finally, 8 male clients as opposed to 6 females fell into the literate category.

The information collected in this section showed that there tend to be consistency in the pattern of utilization between male and female clients in the various categories. It is also discernible that the bulk of the clients for both sexes are concentrated in the semi-literate class. A probable explanation for this seemingly high literacy rate is that Bendel State still remains the oldest in the federation and has enjoyed free education at one time or the other from succeeding administration.

Religion: The religious affiliation of clients formed another focal point of this work. Clients were asked to state their religious affiliation. Their views were tallied under 3 major religious groups: Islam, Christianity and ATR. The results showed that 20 per cent of our respondents were Moslems, 36 per cent Christians and another 44 per cent ATR worshippers.

It will seem from this observation that religious affiliation does not affect pattern of utilizing the services of the traditional practitioners.

Marital status: The marital status of clients is another area in which subjects were quizzed. It was observed that there were 6 males who reported being single as compared to 9 females. Going further, we discerned that 21 male clients were married as opposed to 7 female clients. A male and 4 female respondents fell under the cohort of those separated. There was no divorce figure for male, whereas, 2 was recorded for the female clients.

There is not much to say about the findings for males under marital status except perhaps to mention that in the male category, more married men utilize the services of traditional practitioners than other categories.

Table 2 Number of wives/co-wives

No. wives/ co-wives	Male	Female	Total
Single	6	9	15
1	3	1	4
2	5	1	6
3	6	2	8
4	2	1	3
5	1	0	1
Not reported	5	8	13
Total	28	22	50

Number of wives/co-wives: Another demographic attribute explored in this work, is the number of wives/

co-wives traditional healers' clients have. As before, clients were asked to state categorically, the number of wives in the case of men and co-wives in the case of women. The result is tabulated in Table 2. It is seen from the table that 6 males and 9 females were unmarried. The other respondents for both sexes were married.

Three male clients had 1 wife each while only 1 female client had 1 co-wife. Five males had 2 wives each, while 1 female client had 2 co-wives. Six male subjects and 2 female clients maintained that they had 3 wives and co-wives respectively. Only 2 male respondents and 1 female reported that they had as many as 4 wives/co-wives. Finally, 1 male reported having 5 wives whereas, no female client reported having co-wives to that tone. It will be observed that as many as 5 male subjects and 8 female interviewers refused to report on this variable under discussion. If we add these figures to that reported for the single of both sexes, the left-over will be too few to allow any broad generalization. We shall therefore rest the matter at that.

Table 3 Number of children

No. children	Male	Female	Total
0	4	9	13
1 — 3	2	1	3
4 — 5	3	2	5
6 —10	8	0	8
11—15	4	0	4
16+	2	0	2
Not reported	5	10	15
Total	28	22	50

Number of children: The number of children healers' clients have also constitute one of the demographic variable that was taken into account in this work. This is depicted in Table 3. It can be seen from the table that 4 male respondents and 9 female subjects have no children. Going further, 2 males and 1 female reported that they had 1 to 3 children. Three male and 2 female clients also reported that they had 4 to 5 children whereas, 8 female respondents and no female subject had between 6 and 10 children. Finallyy, 4 males claimed they had up to 11 but less than 16 children while only 2 males had up to 16 and above children. No female client was observed in the last two categories.

It is queer especially at first glance, to see that no female client had up to 6 children. The finding is nevertheless apt considering the fact that we located the modal class for female clients to be in the age cohort 16-30 years.

It is striking that as many as 15 respondents refused to disclose the number of children they had. The explanation for such manifestations lies in the people's world view which forbids the counting of one's children for fear of evil befalling them.

Occupational group: The occupational grouping of clients is yet another major social characteristic that was sifted in this work. The response pattern is as follows: 6 per cent were into farming, 36 per cent reported that they were traders. Four per cent disclosed being engaged in teaching. Another 4 per cent related nursing, 16 per cent were drawn from the class of civil servant, 10 per cent were students while others/uncategorised accounted for the remaining 24 per cent.

It would appear from these findings that in Benin City, traders form the bulk of the clients of the traditional healers. This finding is in order. Moreso, when we recall that as many as 22 males and 18 females fell into the semi-literate category.

At first glance, the 6 per cent recorded by females appears puzzling. Especially as 28 per cent of the clients were illiterates. However, we are less likely to feel such ambivalence if we recall that this research was conducted in an urban centre.

Table 4 Income level

Income per year in Naira	Respondents	% Representation
0—499	1	2
500—999	1	2
1,000—1,499	4	8
1,500—1,999	4	8
2,000—2,499	9	18
2,500—2,999	5	10
3,000—3,499	1	2
3,500—3,999	0	0
4,000—4,499	4	8
5,000+	9	18
Not repoted student	12	24
Total	50	100

Income level: By way of finalising our discussion, subjects were asked to state their annual income. The report was then ranked and presented as in Table 4. We choose here to proceed without order of place. Two per cent of the clients claimed they receive less than ₦500. Another 2 per cent admitted getting more than ₦500 but less than ₦1,000 and yet another 2 per cent professed getting more than ₦3,000 but less than ₦3,500. Similarly, 8 per cent informed the interviewer that they earn more than ₦1,000 but less than ₦1,500; while yet

another 8 per cent claimed they gross below ₦4,500 but above ₦4,000.

We had another tie in the modal category. The first 18 per cent claimed they earned more than ₦2,000 but less than ₦2,500, while the second 16 per cent claimed they generally earn at least ₦5,000. Finally, we had another 24 per cent ranging from students to those who outrightly refused to declare their annual income.

This phenomenon of refusal to declare annual income is usually not unconnected with the fears of respondents, specially among the not too literate group, who probably feel that reporting the exact amount will attract more fines. We are however unable to tell if such factors were at work here.

Section B

Table 5 Age by sex

Ages	Male	Female	Total
1—15	0	0	0
16—30	7	0	7
31—45	10	4	14
46—60	19	9	28
61—75	0	1	1
76+	0	0	0
Total	36	14	50

The characteristics of traditional healers: Table 5 despicts the age by sex of the traditional healers in Benin City. Chronologically, no respondent was to be observed in the age bracket 1-15 years. Only 7 male subjects fell into age cohort 16-30 years. No female was observed in this category, 14 respondents, comprising of 10 males and 4 females fell into age category 31-45 years. Age group 46-60 years represents the modal class. We had a total of 19 males and 9 females in this zone. Finally, while age limit 61-76 years had only one respondent, a female, no interviewer was found in the age grade 76 years and above.

Two comments can be made about Table 5. First, a look at age limit 1-30 years would reveal that only 7 healers are in this category. When these 7 respondents were scrutinized, the researcher discovered that they were slightly under 30 years. It will therefore appear that in Benin City, Traditional healers of both sexes do not commence commercialized practice until they are at least 25 years of age. Secondly, it would appear, especially as no female was observed in this age division that male healers commence practice earlier than female healers in Benin City. It would be fascinating to know if the marital status of female clients has anything to do with this observation.

Education: The educational attainment of healers was one of the attributes that was considered in this work. In going about it, healers were asked to report on the highest level of education they attained. For easy referencing, we divided education into 3 major class as follows: the literate category, comprising of everyone who read beyond secondary class 5, the semi-illiterate class which includes everybody who attained primary 6 but not above secondary class 4; and the illiterate cohort, made up of those who attained below primary 5 or none at all. Their responses were further regrouped in terms of sex. The results show that only 6 male respondents had educational attainment beyond secondary form 5. No female was observed in this category. Going further, 21 male subjects as opposed to one female fell into the semi-literate division. Finally, 9 males and 13 females were found in the illiterate class. From the findings in this work, we can conclude that most practising healers in Benin City today do not have educational attainment beyond secondary 5, and it would appear that in this bid, the female sex are more disadvantaged.

Religion: The religious affiliation of healers formed another area of inquiry in this work. Healers were to state their major religion. When collated, their views led us to propose 3 major religious divisions. This was further expressed in terms of sex. The results showed that 13 out of our sample of 50 healers are Moslems. Closer analysis reveals that they are all males. Similarly, we found that out of 7 respondents who claimed that they are Christians, none is a female. Moving further, we found that of a total of 30 respondents who reported subscribing to African traditional religion (ATR), 16 are males while females accounted for the remaining 14.

This finding suggests that the bulk of the traditional healers in Benin City are believers in ATR. It is remarkable to point out that on further scrutiny, we discovered that none of the 7 Christian-healers employs the art of divinition in their work. They specifically claimed to be herbalists. Conversely, we found that nearly all 30 believers in ATR utilize the divinition technique. It would therefore seem that the religious belief of the traditional healers in Benin City affect their scope of practice and that in this, the Christian-healers tend to be more conservative and have lesser scope of practice, while the ATR-healers have the widest.

Marital status: The marital status of healers formed another area that was scrutinized by this work. As before, we divided marital status into single, married, separated and divorced categories. When tampered with another variables, sex, the response pattern showed

that while 5 male respondents, fell into the unmarried or single category, no female was observed in this group. When expressed in terms of percentage, this male figure comes to 14.1 per cent. We noticed that 31 of the male healers were married. When ranked in terms of percentage, this figure came to 85.9. Again there was no female respondent in this cohort. The reverse is however the case when we examine the cases of separated and divorced categories. It is remarkable to observe that no male healer fell into any of these compartments, unlike 21.4 per cent (3 persons) and 78.6 per cent (11 persons) recorded by female healers under the separated and divorced categories respectively.

From the analysis of this table, it would appear that there are relatively few, if any male traditional healers in Benin City who are unmarried. This finding is supported by the fact that we had earlier located the modal class for male healers in the age category 46-60 years.

Furthermore, it would appear from the absence of female healers in the single and unmarried categories and by the fact of their concentration in the divorced category, that women who are single or who still living under the same roof as their husbands do not engage in practice or commercialized traditional healing. This position is strengthened by the fact that all 21.4 per cent who are still married are separated from their husbands. It would therefore be reasonable, based on this finding to say that the bulk of the practising female healers in Benin City are divorced.

In our consideration of the ages of female healers, we mentioned that it would be worthwhile to see if the marital statuses of female healers have anything to do with their commencing commercialized practice late. In going about this, we ranked the reported marital statuses of female healers by their ages. As before, we tore marital status into single, married, separated and divorced. Age on the other hand was ranked from 1-15, 16-30, 31-45, 46-60, 61-75 and 76 and above. The results showed that no female healer was found under 30 years. We observed that the 3 who fell into the separated category also fell into the age cohort 31-45 years. Of the remaining 11 healers who fell into the divorced category, 10 fell into age grade 46-60, while one fell into age bracket 61-75 years.

What can be said about this finding, is that it corroborated our earlier assertion that women who are single or still live under the same roof as their husbands do not engage in commercialized practice.

Number of wives: The number of wives traditional healers have constituted another analytic unit of this work. As the results had it, 5 healers were unmarried. Three disclosed that they had one wife each. Furthermore, 7 claimed that they were married to 2 wives each. Another 6 reported being respectively married to 3 wives, while 3 others recounted 4 wives each. Finally, 2 healers relayed being married to 5 wives each. It is remarkable that as many as 10 healers refused to disclose the number of wives they had. By way of cross-checking the entire findings in this section, female healers were asked to state if they had any co-wives either while they were living under the same roof as their husbands or separated. It is significant to point out that they all agreed that they had at least one co-wife. The implication of this report is that it tends to define the marital situation in Benin City as a whole rather than wholly relate to the traditional healers. Moreso when it is not clear if the female healers were married to male healers at the time they were married. But since the traditional healers interviewed in this work also reside in Benin City, then it could be said at least in part, that the report could also be applicabe to them. If this basic premise is accepted, then it will not be a far fetched generalization if in addition to our earlier stance about the male traditional healers in Benin City being married, we further add that they have on the average, at least 2 wives.

Table 6 Number of children

No. children	Respondents	% Representation
0	5	10
1 — 3	4	8
4 — 5	2	4
6 —10	8	16
11—15	3	6
16+	5	10
Not reported	23	46
Total	50	100

The number of children each traditional healer has was another sub-heading that featured under demographic attributes. The response pattern or distribution is shown in Table 6. It is readable from the table that 10 per cent of the respondents had no children. Eight per cent had a range of 1-3 children, while another 4 per cent had 4-5 children, 16 per cent of the healers had 6-10 children each, while 6 per cent had 11-15 children. Another 10 per cent reported having 16 or more children. Again we observed as in the discussion of number of wives, that as many as 46 per cent of the respondents did not disclose the number of children they had.

Table 7 Occupational distribution of traditional healers

Main occupation	Respondents	% Representation
Traditional practitioners (excluding spiritual healers)	40	80
Farming	5	10
Trading	3	6
Others	2	4
Total	50	100

Occupational group: Table 7 shows the occupational distribution of traditional healers. And as the table would reveal, as many as 80 per cent of the traditional healers interviewed engage solely in the art of healing. Ten per cent are into farming, 6 per cent in trading activities and 4 per cent in others.

Unlike the case of the healers included in the 80 per cent, the latter groups regard traditional healing as secondary occupation. It should be pointed out too, that some traditional healers included in the 80 per cent have other avocations. We however did not bother to record these as we were only interested in major occupations. Based on this finding, we can safely say that majority of the people who practice the art of traditional healing in Benin City, regard it as their main occupation. If this claim is authentic, then we have reason to believe that they find the art profitable. Whether this is correct or not will be determined by their reported annual income.

Table 8 Annual income

Income per year in Naira	Respondents	% Representation
1—499	2	4
500—999	17	34
1,000—1,499	12	24
1,500—1,999	6	12
2,000—2,499	4	8
2,500—2,999	2	4
3,000—3,499	1	2
3,500—3,999	1	2
Not reported	2	4
Total	50	100

The traditional healers who formed the samples for this work were all asked to state their estimated annual income. Table 8 shows the distribution. Looking at the table, it can be observed that 4 per cent claimed their annual income was less than ₦500, while 34 per cent reported they earned more than ₦500 but less than ₦1,000. Another 24 per cent estimated that they get more than ₦1,000 but less than ₦1,500, 12 per cent agreed that they grossed more than ₦1,500 but less than ₦2,000 while 8 per cent maintained they netted more than ₦2,000 but less than ₦2,500, 4 per cent attested to the fact that their annual income was greater than ₦2,500 but less than ₦3,000. Only 2 per cent reported that their annual income was greater than ₦3,000 but less than ₦3,500. And while 2 per cent testified earning between ₦3,500 and ₦3,999, another 6 per cent related getting above ₦4,000. Finally, 4 per cent refused to disclose their annual income.

Discussion and Summary

In this section, we shall attempt to revisit some of our earlier findings in the hope that more lasting conclusions can be made about them.

In our discussion about the sex of the clients of traditional healers, we discovered that more males disclosed that they utilize the services of healers and were consequently sampled. This result appears queer but is not unique. When confronted with the same findings, Oke (1982) explained that there is a tendency among Yoruba women to report less on this issue for fear of being prosecuted. Whether such factors were at work in this study is difficult to say, but suffices to say that as Africans, a lot of grounds exist for this kind of practice.

In our consideration of the ages of clients, we observed that nobody under 15 years was interviewed and we gave a seemingly plausible explanation for that occurence. Such stance is not supported by Uyanga (1979) who discovered in his investigation of the native doctors in his area of study that children and married women form the bulk of the clients.

Furthermore, we observed that no significant differences exist in the pattern of utilization among age brackets 16-30, 31-45 and 46-60 years. This finding is amply supported by Oke (1982) who concluded after a thorough study of 200 clients that adulthold rather than age per sex was the all important factor when considering the utilization and contact with traditional healers.

Our study of the educational background of clients did not show any significant relations in the pattern of utilization among the literate, semi-literate and illiterate cohorts. This finding is not wholly supported by Uyanga (1979) who discovered that illiterates form the greater proportion of the clients of native doctors he interviewed; whereas, educated persons form the majority of the clients of the spiritual healing homes he sampled.

We observed in our consideration of religious affiliation of clients, that no significant gap exists in the form of utilization of the services of the traditional healers. A seemingly rational explanation for this is that Bendel State is renownedly peopled by a multiplicity of ethnic groups each of which has it's own belief system. We know from the assumptions of culture area theory that a trait usually diffuses outwardly from the concentric centre only after the latter is suffused. If this submission is constant, then it will not be an over generalization if we take the standard of utilization of the services of the traditional healers among the three listed religious group as the cultural trait and assert that having lived together in the same geographic region for a number of years, there tend to have developed feelings of oneness that may have informed this indiscriminate or diffused system of utilization.

The findings in the female category under marital status deserve some comments. The figures appear spurious and sharply contradict our assertion that female clients reported less than their male counterparts for fear of persecution. If one projects this argument, one expects the divorced women to report more than their married counterparts. The results of this work did not however indicate this. Perhaps divorced women have less at stake than their married counterparts. We are however unable for want of date to comment on the extent and nature of relationship under which such an assumption holds.

The findings in this section did not validate our working hypotheses:

1. The higher the educational attinment of a person, the lesser the utilization of the services of the traditional practitioner.
2. The higher the annual income of a person, the lower the level of utilization.
3. Divorced female clients utilize the services of the traditional practitioners more than married female clients.
4. African traditional religionists utilize the services of the traditional practitioners more than Christians.

The implication of this is that in Benin City, the utilization of the services of the traditional practioners is irrespective of social classes. This finding tallies with that of Oke (1982) among the Yoruba and is further complimented by Jahoda (1962) who concludes that the fact that the traditional healer enjoys the patronage of the educated class is vital in accounting for his presence in our midst.

It is more than likely that the real factor at work is the undifferentiated nature of African thought system which links illness to other aspects of social life and subsumes the latter under one causal fabric of man, nature and social relationships. It is such thought pattern which belies the psychosomatic conceptualization of disease and attending illness behaviour of Africans (Chilivumbo, 1976; Namboze, 1983; Crain, 1975; Pfifferling, 1976; Worsley 1982).

Our consideration of the annual income of healers showed that 62 per cent of our sample stated that they grossed less than ₦1,500 per annum. This finding is spurious and fails to tally with the fact that most practitioners take traditional healing are as vocations. Besides, it negates the fact that most of them eagerly reported that they find their practice profitable. Our thinking is that healers under-reported on this variable. This is even more obvious by the fact that most of the healers interviewed live in at least 2 rooms. If we take the average rent per room to be ₦25, then it is difficult to see how they can afford to live on such a paltry sum. This thinking is further supported by the fact that one practitioner whose ₦3,000,000 tradomedical centre has reached an advanced stage, reported an annual income of one Kobo. Couple this with the fact that healers confirmed that they charge at least ₦500 for cases involving mental patients and other serious ailments like sterility, then it will be clear that something is missing.

This finding is supported by that of Erinosho and Ayorinde (1985), who concluded after an extensive work on the traditional practitioners, that they find their practice profitable.

When respondents refuse to report on annual income, it usually arose from mistrust and illiteracy. Whether such explanation can be made in this work is difficult to say. Moreso, when we discovered that the bulk of the practitioners in Benin City had one form of schooling or another. Perhaps their responses were shaped by the current crime wave. It is just probable.

Our analysis of the age by sex of respondents revealed that most traditional healers in Benin City were within the age category 46-60 years. We observed too that this age cohort was closely followed by 31-45 years. Combined, they accounted for a total of 74 per cent of our sample. The implication of this is that most of the traditional healers in Benin City are fast aging. It is our thinking that unless something is done to tap their knowledge, we may never be in a position to benefit from their stock of knowledge less alone transmit them to posterity. The magnitude of this further dawns on us when we realise that 80 per cent of Nigeria's estimated 80 million people seek help from the traditional healers

(WHO, 1978).

In our analysis of he level of education attained by traditional healers, we found that unlike the male respondents who had their modal class located in the semi-literate category, the females had 92.9 per cent of their respondents falling into the non-literate group. The first impression one gets about this finding is that it is spurious. It is possible to account for the figure recorded by the male healers by claiming that Bendel State as the oldest in the Federation, has had succeeding governments offering universal free education at one time or another. Such explanation does not tell us why the female did not take advantage of it. The only seemingly plausible explanation for this phenomenon is that there used to be a belief among the Edo speaking people that it is wasteful to educate female children. The on-going women liberation by our women folk is a part of the moves made to disabuse the minds of the populace about such beliefs.

While considering the marital status of healers, we noticed that the bulk of the male healers were within the age cohort 46-60 years, and were married. The fact that no male healer was fecorded under separated and divorced categories may in part be accounted for by the fact that the healers interviewed for this work generally live in the city. One way they can earn respect and possibly attract clients, especially those of the opposite sex, is to show that they are responsible men. Another is age. The Edo amongst whom this work was carried out, frown on a situation in which a man deliberately decides to remain unmarried long after having reached fecundity. Yet another reason may be seen in the fact that women prove a great helping hands especially with household chores. Such services generally liberate husbands from such tasks and thus permit them to devote more time to their works.

Conversely, we found that most female healers were above 45 years old and divorced. Again, the reason for this lies in the cosmology of the people. Among the Edo speaking people, it is believed that such a phenomenon as menstrual cycle in women lessens the degree of interaction between the people of the corporeal world and those of spiritual. If one took the average menopause age of the African woman to be somewhere between 34 and 45 years, it would appear that there is ground for this explanation. For while the female healers who fell within age cohort 35-45 and who live separated from their husbands may be given benefit of doubt and hence patronized, the same may not apply to female healers of lesser age bracket as it raises the question of their efficiency and positively jeopadize the degree of patronage they are likely to enjoy.

Such cosmogenic explanation was also found at work when in the deliberation on the number of wives and children, we discovered that many respondents failed to report on how many of each variable they had. The Edo forbid people to count the number of children or wives they have for fear that evil might befall them. This phenomenon has also been reported by Oke (1982), among the Yoruba. It would seem from these findings, that the practice of traditional healing in Benin City, is still very much regulated by the traditional beliefs of the people.

The findings in this work have demonstrated that most myths about the person and work of the traditional healer cannot stand to any considerable degree of scientific scrutiny. In his work on the traditional healers' associations among the Yoruba, Oyebola (1981), held the inability of one of his respondents to take blood pressure as necessary indication of impotance to cope with hypertension and cardiovascular diseases. It should be acknowledged that even within allopathic setting, the figure, one out of a sample of 156 would still have been statistically too insignificant to allow such a generalization.

The real issue however, is that even though both the practitioners of the orthodox and traditional healing systems have a common goal in mind and may in some cases serve the same population in needs as Good (1979) rightly observed, they differ in terms of availability, quality of care, technology and social adaptability. Indeed, the ability to take blood pressure is merely a means rather an end in itself. Therefore, the presupposition that the tradomedical practitioner must acquire this ability denies the efficacy in the argument about the inventive nature of mankind, confuses means with ends, as well as fails to acknowledge that diagnosis, treatment, prognosis and the methods adopted are a product of a people's immediate environment and global conceptualization.

Also to be suspected, is the emphasis on empirical verification. It would appear that most analysts gloss over the fact that illness in Africa is a matter of social relationship and that African conceptualization of the aetiology of disease goes beyond symtomological treatment. It is a recognised fact that even within the field of pure science, beliefs about the existence of such phenomena as neutrons and viruses thrive despite our inability to see them with our naked eyes. If this does not shake our belief in their existence, then it is only logical to see the claim of the aura of mystery which surrounds the work of he traditional healer as a diversion. Indeed, as

Chilivumbo (1976), rightly observed, the cure that is meaningful to the patient is that which sets his mind at rest, frees him from his anxieties, reinstates in the patient will to live and restores him to a normal functioning within a therapeutic community.

We discovered in the cause of this work that the labels: 'extortionist', 'clever rascal' and 'Jack of all trades' cannot be said to refer to the bulk of the traditional healers in Benin City. This observation is corroborated by the work of Erinosho and Ayorinde (1985) who while noting the 'deep-seated suspicion' in which western trained doctors hold the traditional healers - because of what they called 'the teaming patronage' the latter enjoy-submitted: "……a very significant proportion in the society had greater confidence in the therapeutic skills of the traditional healers".

On the question of the often made mistake of categorising witch doctor and witchcraft as the same person, Torrey (1972) observed that such a relation is not a relationship but a colonial imputed semantic association. He further argued that all cultures have people who play the role of doctor or healers and most cultures too have the people who play the role of a witch. The same argument can be made in the case of the allusion to the traditional healer as a 'clever rascal' and an 'extortionist'.

Mbiti (1976) has maintained that many trademedical practitioners in Africa are deeply involved in the maintenance of social order and in preserving cultural institutions. This argument has been strengthened by the works of scholars like Ademuwagun (1969) and Ndeti (1976).

Two ways to end this suspicions and to disabuse the mind of the populace is to undertake more researches and to inculcate in the curricula of medical students in tropical Africa, a knowledge of our cultural melieu. Sempebwa (1983) has also made this call.

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シンポジウム報告

欧米先進諸国における熱帯医学教育機構

板倉英幸

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長崎大学熱帯医学研究所（1992a，b）は，1942年（昭和17年）に旧制長崎医科大学に附設された，東亜風土病研究所をその前身としており，1992年（平成4年）に創立50周年を迎えた。その記念事業の一環として，「欧米先進諸国における熱帯医学関連研究所の現状とその展望」と題する国際シンポジウム（Institute of Tropical Medicine, Nagasaki University, 1992）を開催した。近年世界情勢は，東西関係の崩壊，南北問題の複雑化，民族問題の勃興，それに政治，経済問題等が絡み，極めて多様化しつつある。

途上国の多くが占める，熱帯地域における国際情勢も例外ではなく，困難な諸問題に直面しつつ，目まぐるしい変化を示している。このような状況のもとに，欧米諸国の著名な熱帯医学関連研究機関における代表的研究者に，どのような現状認識と，将来展望を持って活躍中であるかを発表と討議をして頂き，我々の今後の実践の場に生かしたいと考えた。各演者の発表内容の中から，熱帯医学教育に関する部分について，これまでに把握できた部分を紹介し，末尾に長崎大学熱帯医学研究所で行われている，熱帯医学に関する教育について付記する。

Hans J. Müller-Eberhard（ハンブルグ熱帯医学研究所所長・教授，ドイツ）：

ハンブルグ熱帯医学研究所（正式名称はBernhard Nocht Institute for Tropical Medicine, Hamburg）は，ハンブルグの衛生局（Department of Health of Hamburg）に所属している。感染性熱帯病の患者の診断と治療，および熱帯医学の教育と研究が使命であると考えている。研究所は西アフリカに，野外調査基地（field station）を持っている。1988年以前の40年間，医師のための熱帯医学と寄生虫学の3カ月のコースを，毎年行っていた。しかし1988年に根本的改組を行い，現在では疾患指向の基礎的研究（desease-oriented basic research）を主体としている。

Paul L. Gigase（アントワープ熱帯医学研究所副所長・教授，ベルギー）：

アントワープ熱帯医学研究所（正式名称はPrince Leopold Institute of Tropical Medicine, Antwerpen）は，1906年Brusselsに設立された。1933年に，主な港町であるAntwerpenへ移転された。当時は，植民地省に所属していた。1960年，アフリカにおける旧植民地領の独立とともに外務省に所属したが，後に教育省（Ministry of National Education）に所属するようになった。しかし研究活動領域は，さらに中央アフリカへも拡がった。1987年にはinpatient departmentはアントワープ大学病院へ移管したので，研究所では10ベッドを持つのみとなった。1991年には連邦制の結果，他の大学も同様であるが，地域の教育省（Regional Flemish Ministry of Education and Culture）に所属し，現在は独立した研究所である。所員は240名で，うち38名は学士号（university degree）を持った人々，すなわち医師，獣医，生物学者である。ほかに何人かの，外部研究費による研究員がいる。

以下，教育について述べる。まずはじめに，医師（post-graduate）を対象としたコースがある。このコースは，熱帯において主としてNGOで働く希望者を対象とし，6カ月コースである。これは言語を，FrenchとFlemishとに分けて行っている。受講者の数は過去3年は毎年80名で，うち半数はベルギー人であり，他はヨーロッパをはじめ他の国の人々であった。10年前は，この2倍の人数であった。生物学者や薬学者も，ときには受講者として加えている。2番目に獣医や農学者を対象とした，9カ月のコースがある。毎年20名を採用し，熱帯における獣医学や酪農を教えている。3番目に，看護婦コースがある。これは1年以上の経験者を対象としており，4－5カ間で年2回開いている。言語はFrenchとFlemishのクラスがあり，受講者全部で約180名である。受講者の1/3はベルギー人で，他はヨーロッパ，スイスなどから参加している。4番目のコースは途上国の有資格者，すなわち学士などの学位（degree）を持ち，熱帯現地で活躍する者を対象としている。これには，以下の4コースがある。1）Public Healthのマスターコースで，International Course in Health Development, およびHealth Plannification and Managementが専門のコースである。FrenchとEnglishのクラスがある。10カ月コースで，毎年40名である。2）Tropical Biomedical Sciencesのマスターコースで，目的はdisease controlである。12カ月コースで，毎年20名である。3）Tropical

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長崎大学熱帯医学研究所

この内容は，第17回日本熱帯医学会九州支部大会における，シンポジウム「熱帯医学教育の理念と現実」（1993年1月15日，長崎市）において発表したものの一部である。

Veterinarian Sciences を専門とするマスターコースで，Ph.D.も取れる可能性がある。研究所はその他，途上国におけるいくつかのコースに参画，参加することもある。Burundi や，WHO による，中央アフリカなどにおけるコースがその例である。

専門家の養成に関する問題としては，1）自国人（ベルギー人）の医師の希望者の減少，帰国医師の社会復帰の困難性，看護婦に関しては数の不足および国内における要求が多いこと，2）途上国における仕事(熱帯医学)の，専門的要求に対応できる医師の教育が，十分になされていないこと，3）国外で働くことに対する，自国社会の認識が薄いこと，などがあげられる。

今後の見通しあるいは方針として，以下のことが考えられる。Public health における専門化は，ますます進むであろう。途上国での専門家は増えるが，工業国からの専門家は減少するであろう。Ph.D.などに至るような，教育の高度化に対応できるようにしておくべきである。看護婦に対しては，通常のトレーニングはせず，instructor として活躍できるような，高度の専門的教育（Masters Degree）を目指すべきである。コンピュータを駆使した教育を，第3国でも応用したい。数週間単位のトレーニングコースを，国外で開きたい。輸入病に関する最小限度の教育などは，各大学でやるべきことで，専門的研究機関でやるべきことではない。

David H. Molyneux（リバプール熱帯医学研究所所長・教授，イギリス）：

リバプール熱帯医学研究所（正式名称は Liverpool School of Tropical Medicine, Liverpool）は，Sir Alfred Lewis Jones によって率いられた実業家団によって，1898年11月に設立された研究所である。当時リバプールは第1の港湾都市で，英国の熱帯地域における利権を握っていた。"Tropical Medicine"なる用語はいまや陳腐で，"International Health"なる用語を好む向きもある。当研究所は，University of Livrepool の附置研究所である。大学院課程（postgraduate teaching）を持っている。教育も研究も目指すところは基本的には，tropical health problem である。教科課程は途上国のニーズに応じて，弾力的なものでなければならない。研究室内研究だけでなく，熱帯野外活動の面でも Ph.D.を取得するに至るような，高度な研究活動のトレーニングを行わなければならない。このことは，学生のみならず教官にも要求されるべきものである。教育の質に対する監査と，すべてのコースについての評価がなされるべきものである。それらのコースは今後，地域的にどこでなされるべきかを考えねばならない。我々は伝統的に本国の研究所で行ってきたが，当研究所では地方病が存在する地域に，専門家および専門的技術を移転（輸出）することを考えている。地域特異的な問題に目標を絞れば，経費も有効に，また指導者の訓練にも役立つのである。当研究所ではすでに，インドにおける母子健康コース，スーダンにおける疫学コース，中央アメリカにおける健康情報システムコース（Health Information System）をやってきたし，またザイールにおいて，地域健康コース(Community Health Course）を，言語としては，French を用いて行うコースとして設立しつつある。これらは，トレーニングコースの将来構想となるべきものである。我々の歴史的活動のなかで，もっとも成功したものとして，25年前にバンコクの Mahidol 大学熱帯医学部の創設がある。これは，Prof. Brian Maegraith の将来構想によるものであった。これにより，東南アジア一帯のネットワーク作りに役立っている。そして多くの Ph.D.を，リバプールから出したのである。一般に短期コースは，コストの点でも助かる。ちなみに Ph. D.のコースであると，$100,000かかる。NGO にしても，コース作りのことも是非考えてもらいたいものである。

Kerl A. Western（NIH，国立アレルギー・感染症研究所国際研究部部長，アメリカ），Richard M. Krause（NIH，国立アレルギー・感染症研究所元所長・Forgarty 国際センター顧問，アメリカ）：

NIH の国立アレルギー・感染症研究所（正式名称は National Institute of Allergy and Infectious Diseases, NIH, Bethesda）では，原則として国外での研究活動基金は認めていなかったが，Public Health Service，特に，AIDS に関して1988年から，アフリカとカリブ地方における研究が認められるようになった。トレーニングについては，主として，所内の研究室で研究活動の実践の場において行われている。

Harison C. Spencer（チュウレン大学公衆衛生・熱帯医学部長・教授，アメリカ）：

チュウレン大学公衆衛生・熱帯医学部（正式名称は Tulane University School of Public Health and Tropical Medicine, New Orleans）は，1834年に設立された。当時，New Orleans ではコレラ，黄熱，マラリアなどが存在していた。現在，米国における熱帯医学に関する唯一の学部で，寄生虫学や熱帯病の研究，教育に取り組んでいる。種々の講座（department）やセンター（center）と連携した活動をしている。当学部には5講座(International Health and Development, Cell and Molecular Biology, Immunology, Microbiology, Biostatistics and Epidemiology), Adult and Pediatric Infectious Diseases Sections, 3つのセンター（Primate Center など），それに研究所などがある。学生数は約500名で，うち25%は国外からである。国籍は米国を入れて，62カ国である。Public health ではラテンアメリカ，サハラ以南，特に西アフリカからの保健(health)に関する専門家養成を行ってきた。Peace Corps とも深い関係を持っている。取得できるのは，Master of Public Health and Tropical Medicine (MPH & TM), M.S.P.H., M.S., Ph.D.,それに寄生虫学における Sc.D.などである。当学部ではいまや熱帯医学研修課程（Tropical Medicine Training Programs）に焦点を当てているのであって，熱

帯病（tropical diseases）における epidemiology の開設を計画している。特に熱帯医学の医学生に，English または Spanish で public health の実習（practical training）を行う。これには，American Society of Tropical Medicine and Hygiene から certification が与えられることになっている。寄生虫学における基礎科学コース（Basic Science Graduate Courses in Parasitology）では，古典的寄生虫学と分子，および細胞生物学と合体したようなものを教える。教育に関しては，tropical health と医学的に重要な寄生虫の laboratory diagnosis の課程の増設を目指している。

いずれにしても，熱帯医学が生き残るには，継続的な学生の確保，および継続的な研究費の確保が大切である。それには，賢明な教科課程や研究課題の設定，熱帯地域途上国の研究機関との継続的連携，他の多くの医学分野との連携が必要であろう。熱帯医学は，常に変化しつつあることを認識すべきである。

Howard D. Engers（WHO マラリア免疫学委員会役員，スイス）：

WHO・TDR（正式名称は UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases: TDR）は，1975年に設立された。次の2つの目的がある。1）途上国で実現し得る熱帯病の診断，治療，予防の方策の開発，および2）医学生物学および社会科学のトレーニングと，研究機関の協力のもとに，医学上の新しい技術開発の研究の強化である。必要性と指導性の2つが，指針である。WHO・TDR では次の6大疾患を指定している。すなわち，マラリア，住血吸虫症，オンコセルカ症を含む糸状虫症，トリパノソーマ症，リーシュマニア症，癩である。研究費の確保からも，研究にしても教育にしても，国内および国際的協力が必要であると考える。

相川正道（ケースウェスタンリザーブ大学医学部教授，アメリカ）：

熱帯医学に関する研究と教育について，日米を比較して見たい。1）熱帯医学関連教育・研究施設（educational system）についていえば，米国では主として私立の大学で行われ，school of public health に併設されている。だから，より自由な政策をとることができる。日本では国立大学が主で，熱帯医学研究所（これはただ1つだけであるが）および，個々の大学の個々の教室で研究，教育がなされている。ただし米国におけるよりも，安定した予算を得ることができる。2）政治，経済上の理由から，米国は歴史的に熱帯医学に関与してきた。熱帯医学に関する教育機関は school of public health として，米国内だけでなく外国からの学生も参加してきた。3）予算の面では，米国では政府とともに，民間からの grant によっている。政府関係機関としては，NIH, NSF, AID, US Army R & D Commands などがある。多くの研究費（research grant）は3年契約なので，長期研究計画が立てにくい。研究成果が不可欠なので，多くの論文が出される。日本では政府からの基本的な予算があるので，長期研究計画を立てやすい。4）一般社会的環境（sociological influence）についていえば，米国における外国からの留学生の多くは，最初は戸惑うが，やがて米国社会に溶けこみやすいようである。日本は単一民族なので，留学生は社会に溶けこみにくいようである。国際情勢から，日本も熱帯地域に自衛隊を派遣するなど，将来はもっと熱帯医学に興味を持つようになるだろう。

演者は，現在ケースウェスタンリザーブ大学医学部（正式名称は Institute of Pathology, Intrenational Health Institute, Case Western Reserve University, School of Medicine, Cleveland）に在職しているが，熱帯医学教育について，国際的な立場で見ると，我が国と西洋諸国とでは，現在までのところ，かなり状況や立場が異なるようである。

長崎大学熱帯医学研究所における熱帯医学教育として，受講対象者がクラス，あるいはグループ形式で行われているものには，次の2つがある。文部省特別事業費による熱帯医学研修課程と，JICA の委託事業の，外国人集団研究コースである。熱帯医学研修課程は，毎年3カ月間，受講定員は約10名，対象者は原則として，医師および生物系の学士であるが，現在では検査技師，看護婦，保健婦等も採用されている。第15回のコースが，平成5年6月に開始される。将来，西欧の熱帯医学研究所のように，近隣諸国からの研修生の参加の道を開くことができれば，さらに盛況を呈することであろう。外国人集団研究コースは，毎年9カ月間，受講定員は約9名，対象者は途上国の医師，生物系の学士等である。第9回のコースが，平成5年1月に開始された。なお東京大学医科学研究所においても，熱帯医学に関する研修課程が行われている。

文　献

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Symposium report

EDUCATION AND TRAINING PROGRAM IN TROPICAL MEDICINE IN ADVANCED COUNTRIES

HIDEYO ITAKURA

Received January 15 1993/Accepted 18 January 1993

On the occasion of the 50th anniversary of the Institute of Tropical Medicine, Nagasaki University in 1992, the international symposium entitled "Present situation and future prospects of institutions of tropical medicine in advanced countries" was held. The symposium was organized by the Institute of Tropical Medicine, Nagasaki University and the Japanese Society of Tropical Medicine, and supported by the Ministry of Education, Science and Culture, Japan.

The current and future topics of "tropical medicine" including present situation, problems, future prospects and educational programs with special emphasis on challenges for the future were discussed at the meeting.

Institute of Tropical Medicine, Nagasaki University

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PROCEEDINGS OF XXXIV ANNUAL MEETINGS OF JAPANESE SOCIETY OF TROPICAL MEDICINE

25-26 November 1992, Nagasaki

President

Keizo Matsumoto

(Professor, Department of Internal Medicine,
Institute of Tropical Medicine, Nagasaki University)

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Prize winner's lecture

JSTM (Japanese Society of Tropical Medicine)
Young Investigator Award

ULTRASONOGRAPHIC AND SEROLOGIC PARAMETERS OF HEPATIC FIBROSIS IN *SCHISTOSOMA JAPONICUM* INFECTION

HIROSHI OHMAE
Nagoya Kyoritsu Hospital and Department of Internal Medicine,
Nagoya University School of Medicine

We described the parameters useful in evaluating the development of hepatic fibrosis in *Schistosoma japonicum* infection, as well as its improvement after treatment with praziquantel (PZQ). Various serologic parameters and ultrasonographic images were examined, and their changes were monitored using rabbits infected with 200 or 300 cercariae of *S. japonicum*. Infected rabbits were administered one oral treatment of PZQ at a dosage of 100 mg/kg at 6, 12, or 24 weeks after infection. Histopathologic examinations revealed that PZQ has a strong effect, even on damage that developed long after the infection. The improvement of moderate hepatic fibrosis that developed over 24 weeks after infection was also detected by histopathologic examinations. The serum level of total bile acid (TBA) was the most sensitive parameter in evaluating the severity of hepatic fibrosis and its improvement after treatment with PZQ. The level of serum procollagen-III -peptide (P-III-P) was also useful in evaluating the development of hepatic fibrosis, but not in its improvement. Ultrasonography revealed specific echogenic bands and nodules according to the progress of granuloma formation and fibrosis, and the reversal of these changes could be observed after treatment with PZQ.

An ultrasonographic examination concomitant with hematologic and biochemical serum analyses was perfomed on 102 patients at the Schistosomiasis Hospital in Leyte, the Philippines. The ultrasonographic liver images were classified into 4 patterns, according to the periportal fibrosis and the patterns of echogenic band. Eleven cases with a long-term infection showed typical septal formation (network pattern). Other ultrasonographic changes in the portal system, such as the severity of splenomegaly, did not correlate with the age of the study patients or the duration of their infection; however, the production of collateral vessels was clear in the group of older patients. Among various hematologic and biochemical serum indicators of liver damage, the serum levels TBA and P-III-P strongly correlated with the development of hepatic fibrosis and portal hypertension.

These patients were subsequently treated with PZQ (3×20 mg/kg), and 52 patients were followed up every three months for a period of 17 months. Improvement in the thickening of the portal vein wall and the intensity of the echogenic bands was detected 6 months after treatment with PZQ. The level of splenomegaly was also reduced in 42 patients who originally did not show the production of collateral vessels. A significant decrease in the serum TBA level was detected in all the patients 6 months after PZQ treatment. However, significant ultrasonographic changes could not be detected in the patients classified as type 3, with severe hepatic fibrosis caused by the long-term infection. These results clearly show that ultrasonographic examination, along with data on the serum TBA level, provides a sensitive tool to monitor the severity of hepatic fibrosis and portal hypertention caused by *S. japonicum* infection, as well as the improvement resulting from PZQ treatment.

Special lecture

THE ROLE OF BIOFILM FORMATION IN THE PATHOGENICITY OF *PSEUDOMONAS PSEUDOMALLEI* AND OTHER RESPIRATORY PATHOGENS

J. WILLIAM COSTERTON
NSERC Professor of Microbiology, University of Calgary,
Cargary, Alberta, Canada

In nature, bacteria grow in two distinctly different modes of growth; planktonic cells float or swim in fluid menstrua, while sessile cells form slime-enclosed biofilms on available surfaces. Recent work in many laboratories has shown that cells in these different modes of growth differ very radically in their structure and physiology, but, most importantly, in their susceptibility to host defence mechanisms and to antimicrobial agents. Biofilm bacteria are protected to a very large extent from antibodies, surfactants, phagocytic white blood cells, bacteriophage, biocides, antibiotics, and adverse environmental conditions such as acidity or drying. The direct examination of bacterial pathogens growing in the human lung (*Legionella, Ps. pseudomallei* and *Pseudomonas aeruginosa* in cystic fibrosis) or in the lungs of domestic animals (*Pasteurella haemolytica* in bovine shipping fever) clearly shows that these cells grow in slime-enclosed microcolonies functionally identical with biofilms.

In controlled animal infection experiments, cells of all four of these bacterial pathogens have been shown to be effectively cleared within four hours when they were administered as free-floating planktonic cells in concentrations between 1×10^6 and 1×10^4, depending on the pathogen in question. However, chronic and occasional acute infections always developed when we administered between 1×10^3 and 1×10^2 cells of the same strains in the form of slime-enclosed mini-microcolonies directly into the lung by aspiration. These data indicated that the biofilm mode of growth of pulmonary pathogens may be pivotal in their ability to establish and maintain colonization in lungs that are defended by specific defence mechanisms such as surfactants, antibodies, and activated phagocytes.

Once established in large microcolonies within the lung, these pathogens can exert pathogenic effects by the release of toxins and by the stimulation of antibody responses that fail to eradicate these organisms, and they may also release planktonic cells from their well-defended microcolonies and thus initiate acute pulmonary infections. It is characteristic of these biofilm infections that they remain indolent for long periods of time, and then flare up to produce overt symptoms. These acute exacerbations often respond to antibiotic chemotherapy, because their causative agents are planktonic, but the protected biofilm niduses are not affected by these agents and these chronic infections commonly recur following treatment with agents that show excellent efficacy against test tube-grown planktonic cells in *in vitro* sensitivity tests.

An unequivocal understanding of the formation of biofilms by bacteria is of great importance in understanding the process whereby bacteria can occasionally establish highly refractory infections of this often-challenged but seldom-infected organ that serves as our respiratory interface with the gas phase of our environment.

Symposium

I. Acute Respiratory Infection

1 GLOBAL STRATEGY FOR CONTROL OF ACUTE RESPIRATORY INFECTIONS

J. TULLOCH
Division of Diarrhoeal and Acute Respiratory Disease Control,
World Health Organization, Geneva, Switzerland

Acute respiratory infections (ARI) are now the leading cause of death in children less than five years of age in developing countries. In 1990 they accounted for 4.3 million deaths, approximately 12,000 per day. They represented 33% of a total of 12.9 million deaths in children. Most of these deaths were due to pneumonia, a large proportion of which is due to bacterial infection that can be effectively treated with antibiotics.

The annual incidence of pneumonia in children under 5 years of age in developing countries varies from 10 to 20 percent, that is 3 to 5 times higher than in developed countries. The higher incidence of pneumonia in developing countries is explained, at least to some extent, by the presence of important risk factors such as malnutrition, low birth weight and nasopharyngeal carriage of pathogenic bacteria which are much more prevalent than in developed countries. There are other risk factors which may also contribute to the burden of childhood pneumonia in developing countries like the prevalence of indoor air pollution from biomass fuel, exposure to chilling in young infants and overcrowding.

The World Health Organization, in consultation with international experts, has developed a simple protocol for the detection of pneumonia based on reliable clinical signs and classification of the disease by degree of severity. The key signs to recognize pneumonia in children are fast breathing and lower chest indrawing. Fast breathing is more sensitive than the presence of rales upon auscultation to detect pneumonia in children. In addition fast breathing is an objective sign which can be taught to paramedical staff, and even community health workers, who can recognize pneumonia in a child in the absence of doctors. Pneumonia is an acute disease which, if untreated, may kill the young child in a very few days, or lead to prolonged or complicated infections.

Streptococcus pneumoniae and *Haemophilus influenzae* are the most common bacterial agents of community acquired pneumonia in children. Standard antimicrobial treatment should therefore be effective against these two bacteria. Cotrimoxazole or amoxycillin are the best choice for treatment of pneumonia, non severe, at home. Essential antibiotics for treatment of severe pneumonia at small hospitals are benzylpenicillin, gentamicin and chloramphenicol.

The protocol was tested in intervention studies in rural areas of eight developing countries; the reduction in the mortality associated with acute lower respiratory infections ranged from 25% to 67%. The protocol is the core of the WHO guidelines on standard case management of ARI in children. Special instructions are given for infants less than 2 months old because the clinical presentation, the etiology and the treatment are different from those for older children.

A series of technical review papers has been issued by the WHO Programme providing the scientific bases for the WHO's recommendations. A wide range of training courses have been developed, or are under preparation. They include training on managerial skills for programme managers and supervisors, and clinical training for doctors, paramedical staff and community health workers. A focussed ethnographic study protocol is available to collect information on the mothers perceptions and practices relating to pneumonia and other ARI in children which is essential for the local adaptation of communication messages. Guides for evaluation surveys at health facilities and at households are being completed. A manual for the surveillance of bacterial drug resistance is being field tested in four countries.

The number of countries with national control programmes was 63 at the end of 1991. WHO is providing technical assistance in planning, managerial and clinical training, communication, monitoring and evaluation. WHO maintains close collaboration with UNICEF in the support to the national programmes.

The WHO ARI Programme also has an active research programme focused on clinical and behavioural aspects of managing ARI in developing countries. A review is being conducted of the feasibility and cost-effectiveness of strategies to prevent pneumonia in children in developing countries.

2 ARI IN THE WESTERN PACIFIC REGION

AKIRA SHIMOUCHI
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Acute respiratory infections, especially pneumonia, are a leading cause of mortality in children under 5 years of age and responsible for some 450,000 deaths annually in this age group in developing countries of the Region. Two thirds, 300,000 deaths occur in China. Main factors of high mortality are (1) poor nutritional condition of children, (2) unavailability of proper case management including proper antibiotics and (3) under-utilization of health services. Generally speaking, in the area where infant mortality is more than 30 per 1,000 live births, ARI account for more than 25% of infant mortality.

Malnutrition is especially prevalent in Cambodia, Laos, Papua New Guinea, Philippines and Viet Nam. In one survey in the rural area in the Philippines showed that more than 70% of children weight less than 80% of standard weight for age.

Mortality is generally higher in rural and remote areas, where often more than half of deaths occur at home without medical attendance. It is because mothers/families do not recognize important signs of severe diseases in time and therefore tend to bring patients to health care workers too late.

Antibiotics are absolutely in shortage in rural areas in Cambodia and Laos and relatively in the Philippines and Viet Nam. In Papua New Guinea supply and distribution of drugs are often irregular, even budget for drugs is fairly sufficient. In those countries, the situation worsens by misuse and overuse of antibiotics for cough and cold. The problem arising in this connection is an increased bacterial resistance to antibiotics, particularly in city areas where overuse of antibiotics is rampant.

In addition even at the first referral hospital (district hospital level) in some countries, proper antibiotics are often not used and intravenous fluids are overused and misused, which may result in a higher case fatality rate yet with high costs.

The objective is to reduce morbidity and mortality from ARI particularly pneumonia in children by introducing prevention and control measures at the community level. Main thrust of the programme is to improve diagnostic skills of pneumonia and to rationalize use of antibiotics. As of June 1992, national programmes have been established in 15 countries including 9 priority countries which infant mortality rate is more than 30 per 1,000 live births. So far 30% of health staff at primary health care level have been trained in the standard case management. A Regional target is that by 1995, 80% of the child population in the Region will have access to appropriate care for ARI. To achieve this target eventually more than one million health staff need to be trained.

Examples of the programme impact are; (1) use of antibiotics have been reduced from 60% to 20% of ARI after 3 years of training and regular supervision in Fiji. (2) The proportion of pneumonia cases which have histories of fast breathing/chest indrawing for 3 days or more had reduced from 62% to 24% after 2 years of face to face health education of mothers at health facilities in Fiji. (3) Use of intravenous fluids and chest X-ray and choice of antibiotics for inpatients with severe pneumonia have been rationalized in the Philippines and Vanuatu. (4) The reduction in mortality from pneumonia has been demonstrated in China.

Reviewing the effects of training programme, regular supervisory visits (for instance, one month, 6 months and 1 year after the training) are found to be indispensable to sustain the knowledge and skills which are obtained by training. Thus a good supervisory activity is a key to a successful programme.

Future emphasis will be as follows; (1) strengthening of health education and supervisory activity, (2) clinical training at district hospital level, (3) undergraduate training for medical and nursing students and students for other health workers, (4) involvement of private sectors by disseminating information and training and (5) securing supply and distribution of antibiotics especially in Cambodia and Laos.

3 ACUTE RESPIRATORY INFECTIONS IN CHILDREN IN THE PHILIPPINES

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Acute respiratory infections (ARI), specifically pneumonia, is a major killer of young children, not only in the Philippines but in other developing countries as well. A program for the control of ARI was thus developed by the World Health Organization in 1982. This program was based on the following assumptions: 1) that high mortality from ARI was due to a high incidence of bacterial pneumonia, 2) that the principal causes of community-acquired bacterial pneumonia were *Streptococcus pneumoniae* and *Haemophilus influenzae* which were sensitive to cotrimoxazole, penicillin, amoxycillin and chloramphenicol, 3) that pneumonia could be diagnosed and the severity of pneumonia determined by quite simple means, 4) that mothers could be trained to recognize the danger signs of pneumonia and 5) that community health workers could be trained to diagnose and treat pneumonia correctly. The Philippine Government in 1979 established the National ARI Steering Committee (NASC) with these specific functions: to correlate and interpret data obtained from research projects and health services statistics, to develop a national programme for the management and control of ARI based on above data and to promote and develop research which is in direct support of the ARI programme. The NASC adopted a program which was directed towards the development of Primary Health Care (PHC) intervention. Research activities to evaluate the effectiveness and feasibility of these interventions in terms of targets set to achieve "Health for All by the Year 2000" were undertaken. It is in this context that I present to you the studies that have been done at the Research Institute for Tropical Medicine (RITM), the research arm of the Department of Health, geared towards the formulation of a national program for the control of ARI with the main goal of reducing mortality due to ARI among children <5 years of age in the Philippines.

Incidence: studies were undertaken to define morbidity and mortality due to ARI and to determine the risk factors for these. From a community-based study in Manila done from 1985-1987, it was shown that Filipino children in an urban population suffer about 6.1 episodes of ARI per year. This is about the same incidence as other children worldwide. However the incidence of ARI or acute lower respiratory infection, specifically pneumonia, is 0.5 per child per year, considerably much higher than in developed countries where the incidence is only 0.03 per child per year. Consequently, mortality is also high. From a study in Bohol Island, we found that pneumonia was responsible for 27% of all deaths in 1,818 children <5 years during a six-year period.

Risk factors for morbidity and mortality: young age (< 2 years), malnutrition, crowding in sleeping quarters, and parental smoking were found to be risk factors for ARI morbidity hospital-based studies conducted in 1984 showed that pneumonia with complications, presence of measles and malnutrition were significant predictors for mortality among children admitted with severe pneumonia. A case-control study of all ARI deaths and their respective controls in Bohol from 1985 to 1991 showed that births attended by traditional birth attendants, presence of low birth weight, non-breastfeeding, non-DPT immunization and low education of mother were significant risk factors for mortality in children with pneumonia. A study on the effect of breastfeeding and the risk of getting pneumonia from 1989 to 1991 showed a significantly higher risk among infants who had not been breast fed.

Etiology: 537 children <5 years of age with pneumonia admitted at the RITM from 1985-1987 were studied. *S. pneumoniae* and *H. influenzae* were found in 42% of 72 bacteremic children. These organisms were sensitive to cotrimoxazoie and amoxycillin, antibiotics recommended by WHO for ARI case management. Yield for bacteria in blood cultures was low partly because of previous antibiotic therapy before admission.

Impact of ARI Control Program on childhood mortality: the ARI control program was developed and implemented in 5 municipalities in the rural island of Bohol (intervention area). Three municipalities did not receive the intervention (non-intervention area). After 6 years of mortality monitoring, a statistically significant reduction in mortality from all causes was shown in both areas but the decline was more rapid in the intervention area (12% per year versus 6%). The reduction in mortality due to primary pneumonia was also higher in the intervention than in the intervention area. This decline in mortality in the intervention area

was very steep during the last two years of the study when intensive health education and supervisory activities were taking place. This activities were very minimal during the first four years and shows the importance of supervision of health care workers for better active case management and also the importance of health education as a component of the control program. A separate study in an urban area showed that health workers in the area were able to comply or adhere to the ARI case management program. Compliance rates or appropriateness of ARI case management improved through time.

4 THE IMPORTANCE AND CONTROL OF ARI IN CHILD POPULATION IN CHINA

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During the last three decades the infant mortality rates were significantly reduced from 250 per 1,000 in 1950s to 40.1 per 1,000 in 1990 in China. Besides economic development and improvement of standard living, there were three important factors of health development related to the reduction of infant mortality improvement of medical services, development of immunization programmes and family planing. With the successful implementation of the immunization programmes, the mortality rates from the vaccine protectable diseases were reduced significantly.

The mortality rated from measles were reduced from 39.8 per 100,000 in 1960 to 0.02 per 100,000 in 1987, similary, polio from 0.16 to 0.004, pertusis from 1.77 to 0.01, and diphtheria from 2.69 to 0.007. The mobidity rated from those diseases were also reduced significantly.

What is the situation of child health after basically control of vaccine-protectable diseases? In order to answer the question, we carried out several epidemiologic studies on child mortality in the areas with different social-economic levels in China in 1990-1991. The data are shown as follows; the IMR and USMR in developed areas, such as in Beijing, were 12.5 and 13.6 per 1,000 respectively, which were quitely close to that in developed countries, however, the IMR and USMR in remote areas and poor areas were still very high. (IMR 40.1-68.0, USMR 49.4-84.4 per 1,000) The studies on death cause in infant mortality suggested that the first important disease causing death in infants and young children is acute respiratory infection (ARI), specially pneumonia. The death from pneumonia accounted for 23.9% of infant mortality and for 22.7% of under 5 children's mortality. While, the vaccine-protectable diseases accounted for only less than 2% under 5 children's mortality in China. Therefore, it is definitely suggested that the first priority in reduction of children's mortality, especially in rural areas is to control pneumonia, which kills about 300,000 children per year in China each year. What we could do for the 300,000 children dead from pneumonia before the new vaccines available to the etiological pathogens ?

Collaborated with WHO and UNICEF, the ARI standard case management was developed and introduced to the national programme of ARI control in China, the purpose of which is to improve delivery of health care for the children with ARI. Three main activities were carried out in study areas, including development of ARI training materials and methods, establishment of supervision systems in primary health level and improvement of facilities of the first referral hospitals in rural areas. The data from a three-year study in three counties indicated more than 50% of reduction of infant and under 5 children mortality rates. Encouraged by the results of the pilot study, the ARI standard case management has been introduced and practised in more than 400 counties since 1991, which covere about 200 million population in China.

5 ARI IN THAILAND AND JAPAN

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It is well recognized that the majority of acute upper respiratory tract inflammations are caused by viral infections which are sometimes followed by bacterial infections. This is generally supported clinically by changes in the initially watery nasal discharge or mucous sputum which become yellowish and prulent. Bacterial infections cause complications of common cold, such as acute sinusitis, otitis media, acute brorchitis and pneumonia. Especially, it is sometimes dangerous for small children and aged people to catch cold because of various above mentioned complications due to secondary bacterial infections.

We have demonstrated in many articles that the most important five respiratory pathogenic bacteria are *Haemophilus influenzae, Streptococcus pneumoniae, Branhamella catarrhalis, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Especially in acute bronchitis and pneumonia, the most important causative organisms are *H. influenzae, S. pneumoniae* and *B. catarrhalis.*

We examined the causative organisms of respiratory infections from 1989 to 1991 at Mae Sot General Hospital in collaboration with Chiang Mai University, Thailand.

We collected sputum from acute bronchitis or pneumonia patients and identified causative organisms with quantitative sputum culture method and inflammatory sputum cytology. The most frequently identified pathogens in respiratory infections at Mae Sot General Hospital were *H. influenzae, S. pneumoniae* and *B. catarrhalis.*

Moreover, we examined the minimum inhibitory concentrations of the representative penicillins, cephems and other antibiotics against these pathogenic strains. In this study, we compared the causative organisms of bacterial respiratory infection between Thailand and Japan.

II. The situation and scope of malariology

1 EPIDEMIOLOGY OF MALARIA: CASES OF NORTH SUMATRA, INDONESIA AND SOLOMON ISLANDS

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Epidemiological studies of malaria is essential for the effective control of malaria. We need baseline data not only of parasitology and entomology but also other sociological data.

Spleen rate has been used as a useful primary index of malaria endemicity. In North Sumatra, by the use of palpation it was found mesoendemic state of malaria. In Solomon Islands, we tried to use a portable ultrasonic machine and found that children less than 15 years of age showed very high positive rate of malaria when he/she has splenomegaly. It may be used as a screening method for malaria since ultrasonic examination is objective, easy and acceptable for children.

Blood examination is essential for parasite rate figure, however, local situation does not allow to use microscope and Giemsa staining procedures. Instead clinical diagnosis is a common practice in most clinics and health centers. This prevents to provide scientifically sound statistics of malaria epidemiology. Occasional blood collection by staff of local health center was not enough to estimate malaria situation in North Sumatra. We found enough training of microscopists is very important to have correct data of parasite rate in Solomon Islands. We have been trying to examine the usefulness of fluorescent staining with Acridine Orange on ordinary light microscope using interference filters. This can be used in villages by utilizing even direct sun light. It is said this method is suitable to detect low grade parasitaemia.

We have been trying also to develop DNA diagnosis of malaria parasites by the use of PCR (polymerase chain reaction). Double PCR method is found to be more sensitive than microscopic examination for confirmation of malaria infection although it takes about 10 hours. Other methods of DNA detection such as ED-PCR (enzymatic detection) and enzymatic demonstration of hybridization have been tried with promising results. These may be used as epidemiological tools in future.

Seroepidemiological studies have been carried out in both North Sumatra and Solomon Islands. We used ELISA with crude antigen of cultured *P. falciparum*. It was found that if we examine students of secondary school, we can obtain informations of malaria endemicity of different villages from which they come. ELISA can be performed in a large scale and provide data of change in epidemiological state as a mass say after any interventions.

In our study in North Sumatra, malaria is not evenly distributed. At the very begining we attempted to conduct stratified random sampling to assess malaria endemicity. We selected two villages from three geographical strata: mountain area, plain area and coastal area. We found patchy distribution of malarious villages in coastal area facing Maracca Strait.

Another important finding in North Sumatra was that gametocyte carriers were mostly in younger age group especially children under 15. And high density gametocyte carriers were in age group less than ten. We attempted to detect malaria in lower grade primary school children and pre-school children. We used not only chloroquine and Fansidar but we added also primaquine in the hope to interrupt transmission. We could obtain drastical reduction of especially falciparum malaria which lasted fairly long period more than one year. One problem we encountered was G6PD deficiency in red blood cell in inhabitants of North Sumatra and Solomon Islands. We detected 4-5% deficient rate in North Sumatra and rather high rate of deficiency 8-14% in Solomon Islands by Fujii's method which is simple, easy and applicable in the field. We must introduce this method before to use primaquine.

We hope these methods we tried in North Sumatra and Solomon Islands could be applicable in malarious rural villages as one of primary health care approach system.

Recent development of mathematical model of malaria transmission made it possible to use computor simulation study in the epidemiology of malaria. We would be able to forecast the results of any intervention of malaria transmission in future.

2 A PRIMATE MODEL FOR HUMAN CEREBRAL MALARIA: EFECTS OF ARTESUNATE

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Although several animal models for human cerebral malaria have been proposed, none have shown pathological findings that are similar to those seen in humans. In order to develop an animal model for human cerebral malaria, we studied brains of rhesus monkeys infected with a primate malaria parasite, *Plasmodium coatneyi*. Our study demonstrated PRBC sequestration and cytoadherence of knobs on PRBC to endothelial cells in the cerebral microvessels of these monkeys. Cerebral microvessels with sequestered PRBC were shown by immunohistochemical analysis to possess CD36, TSP, ICAM-1, VCAM-1 and ELAM-1. These proteins were not evident in the cerebral microvessels of uninfected monkeys. After establishing that rhesus monkeys infected with *P. coatneyi* can be used as a primate model to study human cerebral malaria, we investigated effects of artesunate on this model. Eleven rhesus monkeys were divided into 4 groups; Group I consisted of monkeys which were splenectomized and were treated with 3 doses (200 mg/kg) of artesunate, Group III consisted of monkeys which were treated with one dose of artesunate and Group IV were controls. Parasitemia of these monkeys ranged from 13.6 to 19.5% before treatment. Twenty-four hours ofter treatment, the parasitemia was reduced to 2.2% in Group I, 0.1% in Group II and 10.6% in Group IV. The rate of sequestration which was 30% before treatment became 0.1% in Group I and II and 2% in Group III. These data clearly indicate that artesunate not only reduced parasitemia, but also reduced PRBC sequenstration in cerebral microvessels. ELAM-1 became negative in Group I after the treatment with artesunate, although the presence of other cytoadherence proteins in cerebral vessels was not altered. In summary, this is the first *in vivo* study to show that artesunate reverses PRBC sequestration in cerebral microvessels in cerebral malaria. By using this animal model, we may be able to evaluate strategies for the development of antimalarials and vaccines to cure and/or prevent cerebral malaria.

3 DIAGNOSIS OF MALARIA —REVIEW OF NEW METHODS STUDIED BY JAPANESE RESEARCHERS—

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Microscopic examination on Giemsa stained blood specimen makes the standard method to give definite diagnositic answer to a suspected patient. A new technique developed by Kawamoto (Nagoya Univ.) using acridine organge fluorescence dye is being evaluated in many researchers. The rapid and simple technique seems of considerable applicable value both in the laboratories and also in the field. The method seems to be applicable in endemic countries of poor facilities. DNA hybridization technique using probes of Plasmodia has become sensitive enough to catch extremely small number of parasites in the blood specimen (Wataya, Okayama Univ.). Supplemental diagnosis trial by immunological test is applicable to highly suspected cases in whom parasites are not detected by the blood examination. Indirect fluorescent antibody test leads to even species identification of causative agent in "virgin infection" so far (Kano, Gunma Univ.). Within one year since the onset of the disease, retrospective malaria diagnosis for radically cured patients is feasible by this technique. The results are useful to decide primaquine administration to the patients with past vivax and ovale

infections to prevent relapse. Cerebral malaria is a threatening complication associated with falciparum malaria. Diagnostic measure to predict cerebral complication is required. Recent reports of animal models for human cerebral malaria looks to be promising in promoting the study. *In vitro* drug resistant test is needed to select effective drugs to falciparum malaria before insitituting an anti-malarial administration. Counting parasites at schizont stage in the culture with varying concentrations of a tested drug makes most crucial part of the test procedure. The counting can be repleced by DNA hybridation technique using an appropriate DNA probe from *P. falciparum* (Waki, Gunma Univ.). Ultrasonographic examination of liver and spleen was attempted with schistosomiasis cases in Leyte (Ohmae, Univ. Tsukuba) The method would be applicable in measuring spleen rate in malaria programme. Every diagnositc tool raised above can also be used in the field survey. However, local conditions, feasibilities, cost-effectiveness should be carefully considered in selecting any technique for practical use in the malarious areas which often can least afford to provide laboratory facilities.

4 PRESENT DRUG INVENTION FOR TRAETMENT OF MALARIA

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Malaria is endemic or sporadic through out most of the tropics and subtropics. One hundred million peoples are infected annually and 1% of them die. The incidence of the disease is increasing to different countries by increasing the chance of travelling throughout the world. The incidence of the resistance to popular antimalarial drugs for example chloroquine is also increasing throughout the world. By this reason, a new type of effective antimalarial drug is desired to be discovered.

Polyamines play an important roles in the proliferation of cellular DNA, RNA and protein synthesis as well as the cell proliferation. In present study, we investigate the effects of polyamine inhibitor on malarial parasite.

Deoxyspergualin (DSG), a potent immunosuppressive agent, was proved as a polyamine inhibitor. We proved that DSG showed antimicrobial activity on some bacterial strains. To find out a new and effective anti-malarial drug, deoxyspergualine (DSG), an immunosuppresive agent and a polyamine inhibitor, was tested on *Plasmodium berghei* mouse model. DSG at dose of 5 mg/kg b.w. and 2.5 mg/kg b.w. was injected in mice that infected with *P. berghei*. The effectivity of DSG was proved by decreased the percentage of parasitemia and spleen weight and prolonged the survival days of infected mice.

Not only *P. berghei*, but also *P. falciparum* was shown an anti-malarial effect on the malarial parasite of *in vitro* culture by DSG.

These findings show that DSG will be one of the candidate for anti-malarial drug.

5 CHEMOTHERAPY OF MALARIA

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Historically, preparations from cinchona bark had been used widely in Europe as a specific malaria remedy from early 17 centry. Antimalarial chemotherapy was rapidly developed in this century; till 1950 the discovery of antimalarial agents such as chloroquine, proguanil, pyrimethamine, sulfonamides, sulfones and primaquine. Chloroquine, the most successful schizontocide, has been extensively used as the drug of the first choice for the suppressive treatment of all species of malaria.

After 1960, however, the development resistance of *Plasmodium falciparum* to chloroquine and other schizontocides has been detected in many areas of the tropics and become the chief obstacle to the modern chemotherapy of malaria. Thus, to guarantee protec-

tion against falciparum malaria, the organization related WHO recentry declared that a variety of investigations concerning the development of novel drugs based on the usage of medicine and drug-combination therapy, the establishment of pharmacokinetics in patients, the development of evaluation methods on drug resistance, and medical care based on pathophysiology should have priority.

On recent progress in the chemotherapy of falciparum malaria, pyrimethamine combined sulfadoxicine (Fansidar) was recommended after early 1970's and mefloquine from 1980'. In some areas, serious cases of the falciparum malaria were given artemisinine derivatives or halofantoline. In addition, the development of resistance of *Plasmodium falciparum* to Fansidar, and most recentry also mefloquine, was reported. However, quinine, new understanding of the effect, and in combination with tetracycline still work well for the above mentioned resistant malarial parasite. The effect of parenteral administration of quinine or its isomer, quinidine on the severe malaria has lately attention. Finally, we expect that currentry investigated agents such as pyridinemethanol, triazine, piperaquine and acridine compounds, also pyronacridine developed in China are completed as superior anitimalarial agents.

6 MULTIFACTORIAL CHARACTERISTICS OF ANTI-SPOROZOITE IMMUNITY

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Protective immunity against rodent, simian and human malaria sporozoites can be achieved by immunization with irradiated sporozoites. A number of experimental findings have provided clear evidence indicating that antibodies and T cells play a major role in mediating this anti-malaria immunity.

The protective antibody response has been shown to be directed mostly against the CS protein, located on the sporozoite surface. This protection against sporozoites can be achieved by a) passive transfer of monoclonal antibodies against the repeat domain of the CS protein and b) immunization of mice with synthetic peptides, designed to induce anti-CS protein antibodies.

To enhance the induction of antibodies, the use of potent adjuvants and the modification of the molecular structure of the synthetic peptides, as immunogens, have been performed. Of those improvements, multiple antigen peptide (MAP), which is chemically defined synthetic polymers containing B and T helper epitopes against the CS protein, has been shown to induce high levels of anti-CS antibodies and confer protection against viable sporozoites.

The presence of T cell-mediated mechanisms in anti-sporozoite immunity has been suggested by earlier studies indicating that μ-suppressed mice can mount a protective immunity after sporozoite immunization. Among T cells, the anti-parasite role of cytotoxic $CD8^+$ T cells has been clearly demonstrated in two of the rodent malaria systems, *Plasmodium berghei* and *P. yoelii*. It has been shown that protection induced by sporozoites can be abrogated by depleting the immune mice of their $CD8^+$ T cells. More importantly, recent studies have shown that $CD8^+$ T cell clones directed against a defined cytotoxic epitope of the CS protein conferred protection against malaria, when passively transferred to naive mice.

As for $CD4^+$ T cells, while they have been known to play a helper role in the induction of protective anti-sporozoite antibodies; there has, until recently, been no evidence indicating that $CD4^+$ T cells may also inhibit the development of malaria liver stages. Our recent studies demonstrated that adoptive transfer of $CD4^+$ T cell clone into mice confers protection against a sporozoite challenge. The antigen recognized by this T cell clone is shared by sporozoites and blood forms of *P. berghei*, and differs from the CS protein. The studies of another group also showed the protective activity of $CD4^+$ T cell clones, which recognize the CS protein.

Thus considerable knowledge have been gained, in recent years, regarding the multifactorial characteristics of anti-sporozoite immune mechanisms. The approach of dissecting every components of this immune mechanisms, one by one, will certainly help us to unveil the complexity of anti-sporozoite immunity.

The fact that sporozoite-induced immunity seems to be multifactorial should not be seen as yet another

hurdle for the development of an anti-sporozoite vaccine. On the contrary, it opens the possibility of developing subunit vaccines designed to stimulate different arms of the immune system, which, individually or in combination, may induce firm protective immunity.

Genaral presentation

1 SEROLOGICAL DIAGNOSIS OF DISSEMINATED GONOCOCCAL INFECTION

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The present study was addressed to evaluate the efficacy of serodiagnosis for disseminated gonococcal infection (DGI). Serodiagnosis was done using the purified gonococcal antigen (G-Ag 6.8) which had been determined to be associated with cytoplasmic membranes, and carried out by both the passive hemagglutination test (PHA) and enzyme-linked immunoadsorbent assay (ELISA). Cut-off titers of these two tests were 4 ($\log_2$ titer) for PHA and 30 corrected untis (Ecm) for ELISA, and these tests detected predominantly IgG antibody to G-Ag 6.8 antigen used in this study. A total number of subjects with a tentative diagnosis of DGI was 42 (12 males, 30 females). All of these patients had a past history of urogenital gonococcal infection.

Serological test, either PHA or ELISA disclosed that all the patients were positive with the antibody responding to G-Ag 6.8 antigen and also that antibody titers in their sera fell rapidly after the effective chemotherapy. Isolation of *N. gonorrhoeae* was done from the blood or purulent specimens obtained from the skin lesion. Bacterial culture was positive with 22 out of 25 cases tested. These results suggest that both the serodiagnosis and bacterial culture of specimens from the skin lesions can be valuable for diagnosis of DGI.

2 STUDY ON THE ISOLATION FREQUENCY OF MECHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN HOSPITALS IN GUANGZHOU, CHINA

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Numerous reports of methicillin-resistant *Staphylococcus aureus* (MRSA) have appeared in Europe, U. S. A. and Japan. In China, study on MRSA was begun late and only a few reports about MRSA were published. The isolation frequency of MRSA in Guangzhou, their coagulase type, hemolytic activity and beta-lactamase production and their susceptibility to antibiotics were studied.

168 clinical isolation of *S. aureus* collected from four hospitals in Guangzhou City, during the period from January 1991 to August 1992, were tested by the screening culture methods for MRSA and MIC of methicillin which showed more than 16 $\mu g/ml$.

Of all *S. aureus* strains, 23 (13.7%) were identified as MRSA; 8 strains were isolated from sputum, 7 from pus, 1 from blood and 7 from unknown. 82.7% of MRSA strains produced beta-lactamase, and their colonies showed weak or moderate beta-hemolysis on the blood agar. 14 (60.9%) of them were coagulase type IV. As regards the sensitivity of the MRSA against antibacterial agents, a high antibacterial activity was observed with vancomycin and refampicin, and low antibacterial activity with ceftizoxime, cefmetazole and amikacin. Five strains (coagulase type VII) were relatively resistant to arbekacin (MIC: 8 $\mu g/ml$), which has not been on the market in China.

This results showed that the incidence of MRSA in Guangzhou was less than that in other countries. But as the clinical use of third generation cephems has been increasing, it is likely to be isolated more in the near future.

3 CAUSATIVE ORGANISMS AND CLINICAL BACKGROUND OF ACUTE RESPIRATORY INFECTIONS IN MAE SOT AREA, THAILAND

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The aim of our presenting study is to identify the causative organisms and clinical background of acute respiratory infections and to provide the principle of chemotherapeutic design in Mae Sot, Thailand as one of the tropical countries. The investigations of adult outpatients were performed at Mae Sot General Hospital in Thailand for about one month each year from 1989 to 1992. In each study, Gram-stain of the sputum, quantitative culture of the sputum, and nasopharyngeal culture were done for detecting causative organisms. Minimum inhibitory concentrations (MICs) of each pathogen to frequently used antimicrobial agents were determined.

The cultured sputum revealed major causative organisms of acute respiratory infections mainly in acute nasopharyngitis, acute bronchitis and pneumonia were *Haemophilus influenzae, Streptococcus pneumoniae* and *Branhamella catarrhalis*. This results were the same as those in Nagasaki, Japan. The analysis of antimicrobial sensitivity to major pathogens revealed that *S. pneumoniae* was resistant to tetracycline, gentamicin, and norfloxacin. *B. catarrhalis* showed high resistance against penicillins, 1st and 2nd generation cephems (penicillin G, penicillin V, amoxicillin, piperacillin, cefazolin and cefotiam) and also against tetracycline. In addition, amoxicillin combined with clavulanic acid (β-lactamase inhibitor) showed excellent antimicrobial activity against *B. catarrhalis*. These data strongly suggested that resistance of *B. catarrhalis* might be due to the production of β-lactamase.

From these results of sensitivity, penicillins were recommended as the drug of first choice for *H. influenzae* and *S. pneumoniae*. After this recommendation, penicillins were mainly used. On the other hand the use of tetracycline decreased. However for successful chemotherapy it shoud be emphasized that most of β-lactams are not active against *B. catarrhalis*. In addition, tetracycline is not expected for *S. pneumoniae* and *B. catarrhalis* infections. On the other hand, recently developed new quinolones are effective to the infections caused by both *H. influenzae* and *B. catarrhalis*.

This time, the pharyngeal floras, related to pathogenesis of ARI, was compared with those in Japan. In children *H. influenzae, S. pneumoniae* and *B. catarrhalis* were isolated from pharyngeal flora. In adult pathogenic organisms were infrequently detected. The above results were almost same as found in Japan.

4 A CASE OF BORDERLINE TUBERCULOID LEPROSY WELL RESPONDED TO THE CHEMOTHERAPY

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Leprosy (Hansen's disease; Hanseniasis) is a chronic bacterial infectious disease caused by

Mycobacterium leprae. At first, the organism attacks the peripheral nerves, and then attacks the skin and other organs.

In Japan the number of new leprosy patients has been rapidly decreased in nember. In 1991, only 14 patients reported in Japan (in Okinawa 6). But in the new feature, chances should increase for the foreign leprosy patients to enter into Japan.

In this report, a case of borderline tuberculoid leprosy (BT) is reported. The patient was a 74-year-old Japanese man. There is no patient of leprosy in his family. Present history: About two months ago, anular erythema appeared on the abdomen, and a month ago itching sensation appeared on the upper arms. Clinical findings: On the face a grain sized reddish papule and pea sized erythema are found. On the left upper arm, thumb's head sized anular erythema and common erythema are found. On the left elbow, palm sized anular erythema with central healing is found. On the left breast and right back, pea sized reddish nodulesrespectively. And on the abdomen thum's head sized anular erythema is found. Sensory loss is observed at the all eruptions except the cheek. Hypertrophy of peripheral nerves is not observed. Hair loss is not seen. The thermogram shows normal pattern. Histopathological findings of eruption of the abdomen: Subepidermal clear zone is observed. Epitheriod cell granulomas are scattered in the dermis. Laboratory data: Mitsuda reaction shows positive (7×7 mm). Value of PGL antibody shows negative (SERODIA-Lepra, below 16×); NKcell activity 76% (18-40); Subset of lynphocyte in the peripheral blood shows lea 3a/2a 1.49 (1.59-1.94). Persentage of B cell shows 2% (4-13); Nasal and skin smear shows negative; Fite stain also shows negative. Langhans giant cells are ditected. Dense lymphocytes's infiltration around peripheral nerves are found. Medication and process: After two months with the medication of DDS 50 mg per day, the eruptions disappeared and the sensory loss was recovered in about four months.

5 A SIMPLIFIED PROCEDURE TO INCREASE THE SPECIFICITY OF IMMUNOFLUORESCENCE ASSAY FOR LYME DISEASE

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Serodiagnosis of Lyme disease is performed mainly by use of the indirect immunofluorescence assay (IFA) and the enzyme-linked immunosorbent assay. These serological tests can yield false-positive serology due to cross-reactions with other spirochetes. These probrem complicate the diagnosis of Lyme disease. The tests, however, has not yet been standardized.

A commercially prepared absorbent for syphilis FTA-ABS test from culture fluid of *Treponema phagedenis* biotype Reiter made by Japan Liophilization Laboratory, Tokyo, was used in attempts to reduce cross-reactivities of serum. This reagent was chosen for evaluation because it is commercially available and is widely used in diagnostic laboratories. Serum samples were diluted serially with equal volumes of absorbent and diluted samples were then added to the test plates for analyses in IFA. Although false-positive results occured in high rate in sera from healthy persons with no clinical history of Lyme disease, the use of absorbent effectively reduced non-specific binding to Lyme antigens to undetectable levels without significantly reducing titer of anti-*Borrelia burgdorferi* antibodies.

We present here evidence that the use of a commercially prepared absorbent can enhance test specificity. This procedure may be helpful in the diagnosis of Lyme disease in the laboratory.

6 STUDY OF *LEPTOSPIRA* ANTIBODY AMONG DOGS IN OKINAWA, JAPAN

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Rodents are important animal reservoirs for leptospirosis, and recent studies have suggested that dogs may be one of the important sources of this disease. Some cases of human leptospirosis were reported in Okinawa. But there were few reports about investigation of animal reservoirs of *Leptospira*.

From May, 1991 to March, 1992, 750 serum samples from stray dogs, from 7 health centers in Okinawa, were examined for antibody against *Leptospira* by microscopic agglutination test (MAT). It was done with 12 serovars of *Leptospira* antigens. A titer of *Leptospira* antibody, ×80 or more, was considerd positive.

One-hundred out of 750 serum samples (13.3%) showed positive reaction by MAT. The most prevalent serovar was canicola, followed by serovar pyrogenes and javanica. But there were no positive antibody against serovar hebdomadis, bataviae, pomona, grippotyphosa and rachmati. Dogs in the central and southern areas of Okinawa mainland showed a high positive rate of *Leptospira* antibody. Miyako and Yaeyama areas were low. The male dog showed a high positive rate in comparison with the female.

These results suggested that; dogs were important animal reservoirs of leptospira. But serovar of canine leptospirosis is different from human leptospirosis. Clearly, further studies are required with other animal reservoirs in order to prevent human leptospirosis.

7 EPIDEMIOLOGY OF JAPANESE ENCEPHALITIS IN JAPAN WITH EMPHASIS ON THE EFFECT OF VACCINATION

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The Japanese encephalitis (JE) cases and deaths declined markedly in Japan in recent years, although it is still highly endemic in the Western Pacific rice growing regions.

I reviewed current status of JE in Japan and tried to see why the JE patients decreased and to see the effectiveness of JE vaccination as it was confirmed outside Japan, in Taiwan and Thailand using a placebo group.

National health statistics, production of insecticides for use in rice fields and the amount of JE vaccine sold on the market since 1955 were reviewed and analyzed. Over a period of 44 years from 1947 both patients and deaths declined from the peak of 5,196 and 2,430 in 1950 to 55 and 10 in 1990, although JE virus circulation in nature is confirmed each year. A marked decrease of fatal cases in 8 geographical districts is confirmed but south-western Kyushu and Kinki districts still counted 36 and 22 deaths in 1980s. The number of JE patients decreased in revers relation to the increasing production of insecticides for use in the rice field, reducing the population of vector mosquito *Culex tritaeniorhyncus*.

There was a marked reduction in the ratio of fatal cases below 15 years old among total fatal cases: 56.8% in 1950s to below 2% in 1980s. The declining trend started from 1958, 3 years after JE vaccination for children below 12 years was started in 1955. The same trend for those more than 15 years old started about 6 years later. But the declining trend was greater in the younger than in the older, unvaccinated group. The regression coefficient in the two groups were -0.1380 and -0.1051, which were significantly different ($P < 0.05$). The ratio of fatal cases among total deaths became constantly less in the vaccinated than in the unvaccinated group since 1958. These results may be clear indications of the effectiveness of the vaccine. Increasing use of mosquito nets in the window, decreasing number of farming animals and dislocation of pig-

pens from farm houses contributed to cut human mosquito contact.

8 HEALTH CONSULTATION OF JAPANESE STAYING OVERSEA'S DEVELOPING COUNTRIES

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The tropical zone includes wide areas, big population and huge natural resources. In this sense, the tropical zone is a greatly promising object of the culture and civilization of the future world. Now many Japaneses are inclined to go abroad to tropical countries, but there are many problems relating to human welfare and health in tropical countries. Japanese in tropical countries are often suffered from the diseases which Japanese medicical doctors can not understand, and many problems urgently needed to be solved exist in these Japanese staying oversea's developing countries in this year.

9 FEATURES OF INFECTIOUS DISEASES IN BOAT PEOPLE IN OMURA IMMIGRATION CENTER

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We investigated the incidence of parasitic infestation and hepatitis B virus (HBV) infection in people, who were received from May, 1989 to July, 1990 in Omura Immigration Center. 1,386 people have parasitic infestation (66%) among 2,114 Chinese. Identified parasites mainly consist of *Trichuris trichura* and *Ascaris lumbricoides*. There was no significant difference in the incidence of parasitic infestation between men and women. Prevalence of HBV infection in 1,517 Chinese people was 21.8%.

On the other hand, the incidence of parasitic infestation was 58% among 288 Vietnamese. Identified parasites mainly consist of *Trichuris tricura* and hook worm. Positive rate of HBs antigen was 18.5% among 286 Vietnamese.

Recent reports show that the incidence of parasitic infestation and HBV infection is 0.12% and 1-1.5% in Japanese, respectively. Thus, our data of parasitic infestation and HBV infection in Omura Immigration Center support the high incidence of both infections, and imply the current condition of hygiene in China and Vietnam.

10 RESISTANCE OF MICE IMMUNIZED WITH INDIVIDUAL RABIES VIRUS INTERNAL STRUCTURAL PROTEINS TO LETHAL INFECTION

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Mice were vaccinated with recombinant vaccinia virus (rVac) expressing the glycoprotein (G), nucleoprotein (N), phosphoprotein (NS) and matrix protein (M) of rabies virus and their resistance to peripheral lethal infection with street rabies virus was examined. Mice vaccinated with rVac-G or rVac-N developed strong antibody responses to the corresponding proteins and essentially all mice survived challenge infection. Mice vaccinated with rVac-NS or rVac-M developed only a slight antibody response, however, a significant protection (59%) was observed in the rVac-NS-vaccinated mice, whereas rVac-M-vaccinated mice were not protected. Anti-G antibodies were not detected in the sera of mice which had been vaccinated with rVac-N or rVac-NS and survived challenge infection. Passive transfer of anti-N monoclolal IgG prior to challenge resulted in a significant protection, whereas all mice given F $(ab')_2$ were not protected. However, the protection was not complete even with higher amount of antibodies and the 50% effective dose of anti-N monoclonal antibody was approximately 10 times higher than that of anti-G virus neutralizing antibody.

11 DETECTION OF DENGUE VIRUS IN DENGUE HEMORRHAGIC FEVER (DHF) AND DENGUE FEVER (DF) BY USING POLYMERASE CHAIN REACTION (PCR)

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Dengue/dengue hemorrhagic fever caused by 4 serotypes of dengue virus is a significant health in tropical countries. Dengue hemorrhagic fever (DHF) often occurs among children and is sometimes fetal. However, the mechanism of the DHF is not known yet and early laboratory diagnosis is not yet developed.

Recently, we have established a rapid PCR method by whitch identification and serotyping of dengue virus can be done at the same time in a single tube reaction using dengue virus infected fluids (J. Clin. Microbiol. 1991., Vol 29., p. 2107-2110). We applied the methods to clinical specimens of imported dengue fever and dengue hemorrhagic fever patients and viral genome was detected from all of acute specimens (manuscript in preparation).

We carried this system into dengue epidemic area, Nakhorn Phanom Hospital in Thailand, and examined serum specimens of admitted patients. In this paper, we report the efficacy of the system in epidemic area.

We contacted 81 acute dengue cases and PCR positive rate at admission was 45%. The positive rates of day 2, 3, 4, 5, 6 were 88%, 50%, 31%, 11% and 0% respectively. Especially, the group who admitted the hospital on day 2 and had no anti-dengue antibody, shown 100% PCR positives, indicating high diagnostic efficacy of PCR at very early phase of infection. On the other hand, ELISA positive rate at admission was 48%. When we combine both ELISA and PCR results, diagnostic efficacy of dengue becomes 85% at any phase of infection.

No significant differences of PCR positive rate between primary and secondly infection was observed.

12 DISTRIBUTION OF MALARIAL PARASITE AMONG FEBRILE CASES IN SOUTHEASTERN PART OF BANGLADESH

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The distribution of dlfferent *Plasmodium* species causing malaria among the fever cases attending two selected rural health complexes in the south-eastern part of Bangladesh was investigated in this study. The parasite rate was 45.88% among 194 fever cases of which 58.43% were due to *P. falciparum*, 31.46% due to *P. vivax* and 10.11% due to mixed (both *P. falciparum* and *P. vivax*) infection. The highest rate (61.53%) of malarial parasite (MP) was found in 1-4 years of age group. In almost all age groups the distribution of *P. falciparum* was higher than *P. vivax* or mixed infection. The prevalence of MP positive cases were more among males (50.86%) than female (38.46%). In the tribal population the parasite rare (16.13%) was significantly ($p < 0.001$) low than in the nontribal population (51.53%). In the tribal population 80% of the infection was due to *P. falciparum* and 20% due to *P. vivax*. However in the nontribal population 57.14% were *P. falciparum*, 32.14% were of *P. vivax* and 10.72% were of mixed infection. In lower socioeconomic group the MP positivity rate was higher and the falciparum was the predominant parasite.

13 OCCURENCE OF MALARIA IN PASSENGER OF A BRITISH SHIP

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Malaria is one of the most common infectious disease in the world, there are risk of malarial infection in the epidemic area. The ship doctor requested us to help to diagnose malaria. The ship went to Solomon Island and Papua New Guinea for two months about six months ago. Patients had fever or history of fever and some of them had other symptoms, such as headache, nausea, diarrhoea, myalgia etc. The Ship doctor diagnosed malaria in 59 patients and treated them with antimalarial drugs, fansidar™, chloroquin, and other antimalarial drugs. We examined blood smears of 63 patients by Giemsa stain and we had two positive smears of *Plasmodium vivax* (*P. vivax*). The antibody to *P. vivax* and *Plasmodium falciparum* (*P. falciparum*). Two serum samples had high titer of antibody against *P. vivax* ($\times$1,024 and $\times$64) and three serum samples had high titer of antibody against *P. falciparum* ($>\times16$). From this result, we could diagnose *P. vivax* in two patients, *P. falciparum* in three patients and suspected *P. falciparum* ($\times4$) in 8 patients. 48 patients have low titer ($<\times4$) for malarial parasite, we think they may have no malaria. Though the malaria epidemic is absent in Japan, Japanese health personel should be careful about the occurance of malaria in such circumstances, serum antibody testing is an useful diagnostic method for malaria.

14 MALARIA AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN SOLOMON ISLAND

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In Solomon Islands, south west pacific, malaria is highly endemic. After malaria eradication programme in 1970s, malaria control programme has been carried out until now. Still average annual malaria incidence is as high as 2-300/1,000. Various methods of malaria control trials have been conducted including DDT spray, mass drug administration and use of insecticide impregnated mosquitoe net and so on.

For the treatment of malaria, they have problem of grade 1 chloroquine resistance. In the use of primaquine, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a serious problem in Solomon Islands. If the genetic deficiency is severe, spontaneous hemolysis may occur and also on drug use such as primaquine. This prevents the radical cure of *Plasmodium vivax* infection and the use of it in chemotherapeutic malaria control activities.

We examined the prevalence of G6PD deficiency in Solomon Islands by the use of Fujii's method which is simple and suitable for field study. Among 326 middle and high school students gathered from various islands, 47 (14.4%) showed G6PD deficiency in average. Among 632 blood donors in the Central Hospital of Honiara, 53 (8.4%) was found G6PD deficient by cyanmethohaemoglobin method including intermediate type. Overall agreement between the two methods was 94%. Melanesians was the highest group showed 8.6% (50/582) deficiency followed by Polynesians 6.0% (3/50). Twenty six Micronesians did no not show deficiency. Rates of deficiency varied greatly in each islands ranging from 0 to 50%. In more than 50 islanders examined, Santa Ysabel islanders showed deficiency rate of 17.0% (16/94) in students and 24.1% (14/58) in blood donors. Malaita showed 10.7% (11/103) and 4.0% (11/275), respectively. Students from Central province showed 18.8% (12/64) deficiency rate and blood donors from Guadalcanal showed 14.6% (12/82) and from New Georgia showed 11.3% (8/71) deficiency rates.

These rates of deficiency were remarkably high. Fortunately students did not show anaemia with high Hb level of 14.32 (1.85 g/*dl*) in average. However, in the Central Hospital some patients admitted with hemolysis. However, the rates did not correspond the present day distribution of malaria endemicity although in some islands such as Malaita, Guadalcanal and Central province are the major malarious areas. History of habitation in each islands by different ethnic groups and interrelation are considered to be the source of the present day deficiency rates in those islands of anthropological interests.

Moreover, these high rates of G6PD deficiency pose problems in malaria control in Solomon Islands. Single 45 mg dose primaquine is used in the chemotherapeutic control of malaria. To pursue the effect of gametocyte killing of primaquine in the transmission of malaria which is proven to be successful in North Sumatra, we must be careful to avoid hemolytic crisis in G6PD deficient subjects. Although the rate of hemolysis was reported low up to 18% and practically caused no trouble in Thai subjects, we have to examine the individuals of G6PD deficiency in Solomon Islands. Fujii's method using agar gel plate is simple and very useful in the field of malarious areas.

15 DNA DIAGNOSIS OF MALARIA USING PCR TECHNIQUES

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We have developed three DNA diagnosis systems for detection of malaria parasites in human blood using the polymerase chain reaction (PCR) techniques. Our systems are simple and require neither DNA extraction nor radioisotopes. The double PCR is highly sensilive and specific detection system of *Plasmodium falciparum* parasites using two-step PCR. The target DNA sequence used in the double PCR was that encoded by the dihydrofolate reductase-thymidylate synthase (DHFR-TS) gene of *P. faliciparum*. As little as 10 parasites in 10 μl of blood gave a positive band of 226 base pairs (bp) by agarose gel electrophoresis of the PCR products. In the ED-PCR, enzymatic detection of PCR products, we have chosen a part of 18 S ribosomal RNA gene of *P. falciparum* and *P. vivax* parasites as the target DNA. The PCR products labeled with dinitro phenyl (DNP) were detected using enzyme-labeled anti-DNP antibody. Both *P. falciparum* and *P. vivax* pararites were detected without distinction by the ED-PCR. The microplate hybridization is a diagnosis system which can detect *P. falciparum* and *P. vivax* distinctively, using the *P. falciparum*-specific probe and the *P. vivax*-specific probe to detect the PCR products. In this method, the part of 18 S rRNA gene was also used as the target DNA for the PCR amplification.

We studied these three DNA diagnosis systems in the Guadalcanal of the Solomon Islands, where is a highly endemic area of malaria. 101 blood samples from 98 people were examined by the DNA diagnosis systems and microscopy. The results of our systems well accorded with that of microscopic examination. Our systems were also proved to be useful for a judgment of the effects of treatments.

16 COMPLEX II (SUCCINATE-UBIQUINONE OXIDOREDUCTASE: FUMARATE REDUCTASE) IN THE RESPIRATORY CHAIN OF *PLASMODIUM* MITOCHONDRIA

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The mitochondria of mammalian *Plasmodium* have been variously described as cristate or acristate, or as being absent altogether. Over the past decade, more evidence has accumulated to suggest an active role of the intraerythrocytic *Plasmodium* mitochondria and this was confirmed by the establishment of the protocol for isolating the mitochondria from *Plasmodium*. From the biochemical analysis of the purified mitochondria, occurrence of the NADH-fumarate oxidoreductase system which is an anaerobic respiratory chain found in many parasitic animals such as *Ascaris suum* has been shown. In this system, complex II functions as terminal oxidase and catalyzes the reduction of fumarate to succinate (fumarate reductase: FRD). This enzyme complex functions in reverse direction as succinate-ubiquinone oxidoreductase (SDH) in the aerobic respiratory chain

of mammalian mitochondria.

Mitochondrial complex II is generally composed of four polypeptides and appears to be a highly conserved enzyme complex. The largest flavoprotein subunit (Fp) with a molecular weight of about 70 kD contains covalently bound flavin, and the second largest subunit (Ip) with a molecular weight of about 30 kD contains iron-sulfur clusters. The Fp and Ip subunits are hydrophilic and form a catalytic portion of the enzyme complex that transfers reducing equivalents from succinate to water-soluble dyes such as 2, 6-dichlorophenol indophenol (SDH) or from reduced methyl viologen to fumarate (FRD). Two small hydrophobic membrane-anchoring polypeptides with molecular weights of about 15 kD and 13 kD (cytochrome *b* subunit; cybL and cybS) seem to be essential for the interaction between the complex and quinone species. When *Plasmodium* mitochondria was analyzed by Western blotting using anti-*Ascaris* Fp antibody, cross-reacting band with same size as *Ascaris* Fp was observed. This result indicates a presence of fumarate reductase in the *Plasmodium* mitochondria and antigenic similarity in the enzyme complexes between the two parasites. So, we have started to study the fumarate reductase of *Plasmodium* to know the unique properties of the respiratory chain and its energy metabolism. Homology probing by using mixed primers for polymerase chain reaction (PCR) and subsequent sequence analysis were applied to detemine the partial cDNA sequence of Fp and Ip subunits in the fumarate reductase from *Plasmodium*.

17 ISOLATION AND PURIFICATION OF DIHYDROFOLATE REDUCTASE OF *PLASMODIUM FALCIPURUM* USING SYNTHETIC DHFR GENE

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For the rapid invention of anti-malarials, we have developed the system for preparing a large amount of the target enzyme, DHFR (dihydrofolate reductase) of *Plasmodium falciparum*. Our system involves the synthetic *P. falciparum* DHFR gene which is designed for the efficient expression in *E. coli*. When we induced the artificial DHFR gene into *E. coli*, the product with a molecular weight of 27 kD was accumulated in the cell. However almost all of the products were precipitated in the form of inclusion body. Therefore, we solubilized the precipitate with a buffer containing guanidine-hydrochloride and renatured by diluting with a excess amount of phosphate buffer. Thus renatured fraction showed DHFR activity. The fraction was followed by the further purification processes including Hydroxylapatite and Sephacryl S-300 column chromatographies. We obtained 10 mg of DHFR with a purity of more than 98%.

The Km values of our purified DHFR and NADPH were respecticaly 15.0 μM and 19.6 μM. These values for both substrates are similar to those of partially purified DHFR from *P. falciparum* reported by Kan and Siddiqui (J. Protozool., 26, 1979, pp. 660-664), therefore it is suggested that our purified DHFR has the same kinetic properties to the native DHFR.

18 AN ANTIMALARIAL ACTIVITY OF DILAZEP AND DIPYRIDAMOLE

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An antimalarial activity of dilazep and dipyridamole was investigated by using a continuous culture system of *Plasmodium falciparum*. When 2 μM of dilazep or 3 μM of dipyridamole was added to the culture, the parasitemia was reduced significantly on day 4. The inhibitory effect appeared parallel to the concentration of these drugs. The antimalarial activity was also observed when these drugs were added to the culture

containing uninfected erythrocytes 24 hr before *Plasmodium falciparum* infection. These results indicate that dilazep and dipyridamole might act on the erythrocyte membrane, thereby inhibiting erythrocyte invasion by *Plasmodium falciparum*.

19 OVALE MALARIA-LIKE MORPHOLOGICAL CHANGES OF THE PARASITIZED RED CELLS IN TERTIAN MALARIA

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Differential diagnosis of malaria is based on the morphological characters of parasites and parasitized red cells in thin blood smears. Individual immune situations and drugs used can transform malarial parasites. Red cells can also change shapes due to not only congenital and acquired factors but also techniques of blood smear preparation. Therefore, it is sometimes difficult even for specialists to differentially diagnose the four malarial species, especially between *Plasmodium vivax* (Pv) and *P. ovale* (Po), or *P. falciparum* and *P. malariae*. In this study, we examined the frequency of ovale malaria-like morphological changes of parasitized red cells in thin blood films of some tertian malaria cases, because a smear in an Indian Pv case (Pv/Po) with high IFA titer to Pv antigen revealed many parasitized red cells had changed morphologically. As a rule, the first thin blood films from 2 Po, 1 Pv/Po and 16 Pv cases were examined. Morphological changes was classified into three groups, ie. 1) fimbriation only 2) elongation only and 3) both fimbriation and elongation. These were represented as percent frequency. Frequencies of fimbriation only, elongation only and both fimbriation and elongation were 25.6%, 11.8% and 25.1%, respectively in Po. In Pv/Po, these were 34.0%, 7.6% and 5.2%, whereas in Pv, only 1.6%, 0.2% and 0.6%. This result may show that Pv/Po cases are caused by a peculiar Pv species.

20 ANTIBODIES INHIBITING THE ATTACHMENT OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES TO TARGET CELLS

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Three kinds of immunological assays, antibody-mediated agglutination of erythrocytes infected with mature stages of *Plasmodium falciparum* (IRBCs), inhibition of attachment of IRBCs to C32 amlanotic melanoma cells and IFA on the surface of alive IRBCs (surface IFA), were conducted by using IRBCs of several *P. falciparum* isolates and sera from animals immunized with IRBCs and from patients to confirm that these sera contained the antibodies binding to the surface of alive IRBCs.

Sera from eight acute falciparum malaria patients were examined on the assays by using IRBCs of homologous parasites which were established in *in vitro* culture. All eight sera agglutinated IRBCs of an isolate MS804, but no sera inhibited the attachment of them to C32 melanoma cells. A homologous pair, IRBCs of an isolate MS815 and homologous serum, showed a positive agglutination and inhibition. Both of the positive agglutination and inhibition were not found on the other seven pairs. Surface IFA were negative for all the sera. It was suggested that the titers of antibodies binding to the surface of alive IRBCs were low in these acute patient sera.

On the assays serum from a recrudescent patient was compared with the serum taken from the same patient one month before the recrudescence by using homologous parasite isolates. Although the recrudescent serum showed stronger agglutination and inhibition

of IRBCs of both homologous isolates than the one-month-before serum, it showed negative surface IFA as well as the latter serum. A recrudescent serum might not necessarily contain high titers of specific antibodies.

Mouse sera and rabbit sera, which were taken from the animals immunized with IRBCs of strain M, inactivated and treated with human O group erythrocytes to remove antibodies binding to erythrocytes surface, gave positive results to the agglutination and inhibition assays. While rabbit pre-immune sera did not agglutinate M strain IRBCs, mouse pre-immune sera agglutinated them. Surface IFA was negative for mouse immune sera. Rabbit immune sera did not show surface IFA clearly.

Immunoglobulin G were purified from a rabbit serum by using Ampure PA (Amersham) to obtain positive surface IFA. The IgGs agglutinated IRBCs, inhibited the attachment of IRBCs to melanoma cells and gave a positive result to the suface IFA. A quantitative correlation was found between agglutination and inhibition assay titers of the IgGs solution and of the original rabbit serum. While the original serum and the IgGs solution showed almost the same titers in the agglutination and the inhibition assays, the serum without an IgG component showed very low titers in the two assays. Agglutination was detected at higher dilution of the IgGs solution than surface IFA.

21 NEUROPATHOLOGICAL FINDINGS OF BRAINS OF MALARIA TROPICA

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I want, though insuficient data, to report about macroscopic findings of brains and abdominal organs of 45 Japanese soldiers, suffering from acute and serious malaria tropica in Bataan Peninsula battle fields in Philippine, who died suddenly as soon as arrival to hospital and in a few days in spite of sufficient medical treatments in our basic hospital in Quezon City from April to July 1942. These 45 soldiers were all belonged to a newly organized army division in Japanese home province. Accordingly they had no anamnesis of malaria infection. And also two cases in southern China, which given to me from field hospital in 1938, microscopically studied in Neuro-Psychiatric Department of Kyushu University in Fukuoka. These two cases were well and sufficiently treated.

Clinical symptoms were generally high fever in every day, anemia, enlarged spleen, delirious and then comatose condition, convulsion and many kinds cerebral symptoms. At last suffered a loss by heart diseases.

Macroscopically summarized in 45 brains, I observed the two kinds of haemorrhage. *At first*: precapillary and capillary haemorrhages bandlikely arranged between cerebral grey matter and white matter. This localization put us in mind of spongy oedema by Creutzfeldt-Jakob's disease. I remember Pfeifer's "Die angioarchitektonische areale Gliederung der Grosshirnrinde, Leipzig 1940". Small superficial pialartery arriving to the bottom of windings valley, deeply passing through grey matter, spread many rectangular precapillary branches and farther capillary branches rectangulary. This repeated rectangular remification may be locus minoris of haemorrhage. *Secondary*: rather larger haemorrhage in brainstem esp. in pons.

Microscopic findings of one of two cases in China: I saw embolus of capillary and precapillary within malariapigment and lymphocyte included, its surrounding area is chaemic necrosis, fatty soft degeneration and micro-, astro- glial proliferation i.e. *Dürck's Malaria Granuloma*. In white matter of both cases were seen venous stagnations.

Abdominal organs: Enormous darkred spleens were seen just as to burst, maximal size reached $25 \times 10 \times 8$ cm. Liver showed wedge-shaped white infarct, its maximal superficial diameter ca. 5 cm. Kidney also had 2 cm diameter of white-infarct.

22 DEEP VASCULAR SCHIZOGONY IN INTRADERMAL CAPILLARIES IN FOOT PAD OF *PLASMODIUM COATNEYI* INFECTED RHESUS MONKEY —INTRADERMAL METHOD FOR MONOTORING CEREBRAL MALARIA—

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Severity of falciparum malaria in cerebral malaria patients is reported to be not necessarily related to peripheral circulatory parasitemia, but to the degree of parasitized red blood cell (PRBC) sequestration in cerebral microvessels. The sites of deep vascular schizogony by *Plasmodium coatneyi* (*P. c.*) have been investigated by several researchers. And, recently, Aikawa *et al.* established a primate model for the study of human cerebral malaria by demonstrating *P. c.*-parasitized red blood cell sequestration and cytoadherence of knobs on PRBC to endothelial cells in the cerebral microvessels of rhesus monkeys (*Macaca mulatta*). However, obtaining a brain smear for the oxamination of sequestration from a cerebral malaria patient is impossible. And yet, watchful and adequate management requires the cerebral malaria be differentially diagnosed from the other causes of cerebral manifestation, and that progression of severity be closely monitored during the entire clinical course. Therefore, pathological evaluation of the severity of cerebral malaria is still very difficult but necessary. So that we have devised an intradermal method for obtaining the degree of deep vascular schizogony using a *P. c.*-infected rhesus monkey: a spot on the foot pad of the monkey was punctured intradermally at least 10 times using a 27 G needle from different angles, in order to damage the tissues and free schizont-infected erythrocytes from the microvessels. A drop of blood was squeezed out of the spot onto a glass slide. And blood smears taken by this method were contrasted with those taken simultaneously from the ear lobe. It was proved that parasitemia of the schizont-infected erythrocytes observed in the blood smear made by the intradermal method from the foot pad was always significantly higher than that obtained from the ear lobe. And it was also worthy of our notice that the zig-zag pattern of the change of daily parasitemia of ring form-infected and schizont-infected erythrocytes were just the opposite. We could say that the degree of deep vascular schizogony at the foot pad could be very well shown by the intradermal method, and the severity of cerebral malaria could thus be monitored successfully by the method.

23 ROLES OF γ/δ T CELLS IN PROTECTIVE IMMUNITY AGAINST MALARIA

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A number of studies on protection induced by immunization with malaria sporozoites have demonstrated that antibodies and T cells bearing CD4 and CD8 play an important protective role. These T cells, which seem to eliminate malaria parasites by attacking the liver stages, express α/β T cell receptor (TCR).

In order to define the possible role of non-α/β T cell populations in immunity against the rodent malaria *Plasmodium yoelii*, we studied the anti-malaria immune response in mice deficient in α/β or γ/δ T cells. These mice were generated by introducing germ line mutations in either the TCR-α, β (α/β T cell deficient), or δ genes (γ/δ T cell deficient).

When α/β T cell deficient mice were infected with non-lethal strain of *P. yoelii*, they developed uncontrolled ascending parasitemias. Differently, normal

mice were capable of controlling and clearing the blood infection. The course of parasitemia in γ/δ T cell deficient mice was similar to that observed in normal mice. These results indicate that α/β T cells are essential to control the parasite infection of blood cells, and strongly suggest that γ/δ T cells do not have a major protective role against the blood stages of the parasite.

Immunity against the liver stages appears to be different. Immunization of α/β T cell deficient mice with irradiated sporozoites induced an immune response that significantly inhibited the development of the liver stages, indicating that non-α/β T cells have an anti-parasitic activity. In fact, the depletion of γ/δ T cells in sporozoite-immunized α/β T cell deficient mice, after *in vivo* administration of anti-γ/δ antibodies, strongly abolished the protective immunity. These results strongly suggested that γ/δ T cells could mediate the anti-malaria immune response observed in these mutant mice.

Finally, we isolated two γ/δ T cell clones from malaria-immunized α/β T cell deficient mice. Adoptive transfer of one of these γ/δ clones to normal mice significantly inhibited the development of the liver stages of the parasite.

24 BASIC STUDIES ON THE MONGOLIAN GERBIL AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION (10) UMBILICAL SCENT GLAND

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The Mongolian gerbil is one of widely used animals in various fields of experimental medicine, however, detail biological studies on this rodent have been less than those on mice or rats. The Mongolian gerbil has a scent gland on the umbilical skin area and it is one of the peculiar characteristics of this rodent. In this report, we made morphological and histological studies on the gland.

The gland was observed to be thickened in a spindle or elliptical shape on the umbilical skin area. Sizes of the gland were larger in males than in females and in some famele gerbils, it could not be found macroscopically on the ventral skin. The gland was packed in the corium and orifices of the gland were placed in a line to surface of the skin. In some gland, secretion was produced. In some female gerbils lacking the gland in macroscopic observation, absence of the gland was demonstrated by a histological examination.

These findings suggested that the umbilical gland of the Mongolian gerbil was a sebaceous holocrine gland which produced a scent for territorial behavior and related to difficulty of matings.

25 BASIC STUDIES ON THE MONGOLIAN GERBIL AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION (11) SKIN SENSITIVITIES TO CHEMICAL MEDIATORS

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Our previous reports showed the Mongolian gerbil (*Meriones unguiculatus*) had basophilic granulocytes in peripheral blood and mast cells in connective tissues. These characteristics are almost same as those of the

guinea pig among small rodents. In this experiment, we carried out comparative studies on skin sensitivities to some chemical mediators among gerbils, rats and guinea pigs.

Skin sensitivities to chemical mediators such as acetylcholine (Ach), histamine, serotonin (5-HT) and leukotriene C_4 (LTC_4) were compared among male and female gerbils (JMS strain), male rats (Sprague-Dawley strain) and male guinea pigs (Hartley strain) using a modified method of immediate intracutaneous reaction under conventional condition. Each mediators in ten-fold serial dilutions in a volume of 0.1 m*l* was inoculated intracutaneously into sites on the shaved back skins of these rodents. Immediately after intracutaneous inoculations, 0.25% Evans blue in saline was injected intravenously. After 30 min, areas of blue spots on the inner side of the skin were measured and the doses of mediators which induced the same size of blue spots in three rodents were compared.

The skins of Mongolian gerbils appeared to have a high response only to histamine with almost same degree of guinea pigs. The reaction to Ach of gerbils was in the middle level between rats and guinea pigs. To 5-HT and LTC_4, skin sensitivities of gerbils were lowest among these three rodents and areas of reactions were not enhanced with increase of doses of 5-HT and LTC_4 in gerbils. The skins of rats remarkably responded to 5-HT and LTC_4 but not to histamine.

The Mongolian gerbil was appeared to be a histamine sensitive rodent like the guinea pig in this experiment. This characteristics including existence of basophils and mast cells in gerbils may be very useful to study some allergic reactions during the course of filarial infections.

26 BASIC STUDIES ON THE MONGOLIAN GERBIL AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION (11) SENSITIVITY TO EMC VIRUS M VARIANT

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It is well known that encephalomyocarditis (EMC) virus induced diabetic symptoms in mice. In this experiment, we studied on sensitivities to EMC virus M variant of the Mongolian gerbil which widely used as a susceptible host to filarial infection.

Conventional male and female gerbils were allocated into 4 groups at the age of 3-4 months. Three groups of 9 gerbils each were inoculated with EMC virus M variant intraperitoneally (10^2, 5×10^2 and 10^3 $TCID_{50}$/0.1 m*l*, respectively). Gerbils of the other group were injected with medium in the same volume of virus solution as a control. Their food and water consumptions, body weights and blood sugar levels of gerbils were measured during one week after virus inoculation. Blood samples were collected from the tail vein and blood sugar levels were determined by glucose-oxidase method. At the time of sacrifice or death, hearts, kidneys and pancreases were removed and tissue sections of those organs were prepared as usual.

Average blood sugar levels of each virus inoculated group of gerbils dropped momentary at the 3rd day of virus inoculation and after that, it returned to normal ranges. Because blood sugar level did not increase in virus inoculated gerbils, EMC virus M variant could not induce diabetic symptoms in the Mongolian gerbil. Pathological changes of virus inoculated gerbils were severest in pancreas among three organs. Acinar cell vacuolation and fat necrosis were found even in animals inoculated with the lowent viral dose. In gerbils inoculated with the highest viral dose, islet cell hyperplasia was observed. In hearts, atrophy of cardiomuscular cells and cellular infiltration were observed in acute phase and showed signs of acute myocarditis. Kidneys of virus inoculated gerbils were almost normal in acute phase. Degrees of these changes of the tissues were not different between sexes but were in proportion to infectious doses of EMC virus.

This study suggested that the Mongolian gerbil has sensitivity to EMC virus M variant and may be a useful model to analyze relationship between viral and filarial infections.

27 BANCROFTIAN FILARIASIS IN KENYA

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The control project of bancroftian filariasis in Kenya was initiated in May, 1990 under the sponsorship of JICA and Kenya Medical Research Institute. The main aim of the project is to understand the epidemiology of the disease and to find out an effective method of control, especially to study the effect of treatment combined with ivermectin and diethylcarbamazine.

According to the preliminary night blood survey, the study areas were settled down at three villages, Lutsangani, Dzivani and Gandini, 90 km west of Mombasa.

The present paper deals with the basic epidemiological survey done at one of the study areas, Lutsangani, on Februry, 1991, November, 1991, January, 1992 and June, 1992.

Although the census of the study area has not been completed, the population of Lutsangni is estimated to be 1,100. The villargers over 5 years old were requested to receive the night blood survey and clinical examination. A total of 436, 233 males and 203 females, were examined for microfilariae by nucleopore filtration method. The microfilariae were detected in 86 or 19.7% of subjects examined. The higher mf rate was found in persons over 40 years. There was no difference in prevalence by sex. The micrifilariae density varied from 1 to 2,944, with the mean of 357.5.

A total of 341 villagers, 181 males and 160 females, were examined by doctors. Twenty eight of them, 14 males and 14 females, showed one or more of acute and chronic signs of bancroftian filariasis. For males, epididymitis and hydrocele was most common. They were found in 12 and 11 persons, respectively. For females, lymph varices was most common and it was found in 11 individuals. Elephantiasis was found in 2 males and 2 females. Chyluria was absent.

Entomological survey is now under investigation.

28 DEVELOPMENT OF THE NEW TYPE VACCINE AGAINST THE FILARIAL INFECTION 1. EVASIVE MECHANISM OF FILARIA FROM HOST IMMUNE SURVEILLANCE

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We carried out the experiments of host immune response to the filarial infection for past 25 years. We could clearly the mechanisms of the clever evasive mechanism of the filarial worm from host immune surveillance. Now we intended to develope of the new type vaccine against the filarial infection after understanding the evasive mechanism of the filarial worm. The new type vaccine is supposed to contain some elements of (1) antibody against the 3rd stage larvae (L3), transmission blocking antibody, (2) activate substance of the effector cells such as macropharge and eosinophil and (3) of the NK cells activate substances.

29 DEVELOPMENT OF THE NEW TYPE VACCINE AGAINST THE FILARIAL INFECTION 2. TRANSMISSION BLOKING VACCINE

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Effector cells in filarial infection were determined to be macrophages and eosinophils. And these effector cells worked only to the 3rd stage larvae (L3). We have produced monoclonal antibodies raised by *Brugia pahangi* L3 antigen, and have obtained monoclonal antibodies recognizing a stage- and species- specific antigen. When this antibody was given to the *B. pahangi* infected mice, the infection and recovery rates of the filarial worm from the mice were obtained to decrease significantly. The antigen recognized by the monoclonal antibody disappeared from the surface of the 3rd stage larvae within 1 week after infection.

30 MEDICAL EXAMINATIONS OF CHILDREN IN KINGDOM OF TONGA

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We carried out the investigation on the relationship between health and the eating behavior of Tongan.

A comparative survey have carried out to elucidate how the designated concept of "Healthy obesity" prevailed in Tongan adult population, among 3 area, Kolofóou in the capital (K area), Uiha in the isolated island (U area) and Manukau area in Manukau City in New Zealand.

It is reported that we surveyed inhabitant medical examinations of Tongan children. Subjects are consist of 85 children aged 8-17 year.

Children have been compared with the WHO standard in respect of weight/age, weight/height, height/age and body mass index (BMI). Tongan children are heavier than other population.

In Urinalysis, albuminuria and glucosuria were not detected. Hematocrite test in blood examination showed the range of 37-52% in males and the range of 32-42% in females.

As to fourteen items of blood chemistry, the significance of difference between K area and U area was assessed on the basis of Student's t-test. In males and females, there were a significant difference in GOT and GPT between K area and U area, respectively.

We studied for serological evidence of hepatitis B virus markers. As to the results of HBV markers, 16 out of 85 subjects (19%) were found to be positive for HBsAg, and 45 out of 85 subjects (55%) had Anti-HBs.

31 A CASE STUDY OF INTERNATIONAL TECHNICAL COOPERATION IN THE FIELD OF TROPICAL INFECTIOUS DISEASES IN NORTHEAST BLAZIL

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For the last 6 years, we have been participating in the international medical cooperation project on the Interdepartmental Center for Immunopathology (now called LIKA-Laboratorio de Imunopatologia Prof. Keizo-Asami) in the Federal University of Pernambuco, supported by Japan International Cooperation Agency (JICA). It followed one of the scheme of JICA called "Project Type Technical Cooperation". The Northeast is one of the least developed regions of Brazil and also an endemic area of many tropical infectious diseases. The project started with its objective "to improve the research on tropical parasitic diseases at the Center and to contribute to the better hygienic condition in the Northeast Brazil". During the course of project, it became necessary to include as the target of research not only parasitic diseases but other infectious diseases. Later, the cooperation was also extended to the area of clinical research, in particular endoscopic and ultrasonographic diagnosis, mainly aiming at schistosomiasis.

During this project, the total number of 70 Japanese experts were sent to the Center, 23 trainees were received in Japan and the epuipments in a total amount of 550 million Japanese yens were donated. As a result of such a cooperation, the research center which started from almost zero has become one of the leading tropical disease research centers in Brazil, with annual publication of more than 20 including those published in international journals. In addition, more than 30 young students have already completed their master's theses through oxperiments conducted at the Center. As these experimental achievements have been recognized, researchers in this center are now able to obtain research grants. In this way, the crucial problem of how to sustain the activities of the research center after the end of cooperation project is being solved.

Through this project, we have detected 3 main problems: (1) The problem of "local cost" bearing, (2) The problem on the objective, evaluation and meaning of the research development projects, (3) The problem on continuity in research development projects after the completion. Based on our experiences, we discuss the following points for each problem mentioned above: (1) The condition of our counterpart institution at the initial stage of the project and our basic strategy, (2) Objective and meaning of funding a research institute in developing countires in special reference to local cost bearing, (3) Trial to obtain research grants from outer foundations.

32 ROTAVIRUS EPIDEMIOLOGY IN ZAMBIA

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An epidemiological study on Rotavirus infection was carried out at the University Teaching Hospital (U. T.H.), Lusaka, Zambia. This hospital has bed space of about 1,500 and is the main national referral hospital in Zambia. It also caters for population of about one million people living within and around Lusaka. Between November 1991 and September 1992, 1,139 stool samples were collected from pediatric patients

admitted to the diarrhea unit of the U.T.H. Stool samples were tested using Rotavirus antigen detection ELISA test kits (Rotaclone, Cambridge Bioscience) and a electron microscope (negative staining). Of the 1,139 samples tested 294 were positive for Rotavirus (positive rate: 25.8%). During study period there was an epidemic of dysentery in Lusaka. Rotavirus positive rate was found to be higher in non-dysenteric cases (non bloody diarrhea) (212/505, 42%) than in dysenteric cases (bloody diarrhea) (44/449, 9.8%). The overall Rotavirus positive rate could have probably been higher, had there been no dysentery epidemic.

Rotavirus positive rate was higher in male (28.2%) than in female (23.5%), but this was not statistically significant. Rotavirus was more common in younger age group, especially those less than one year of age. The positive rate decreased with advance in age as shown below; less than one year: 39.9%, above one year less than two years: 18.0%, above two years less than three years: 15.4%, above three years less than four years: 16.2%, above four years less than five years: 11.1%, and above five year less than six years: 12.0%.

Mortality rate in Rotavirus positive cases was 9.1%. This is higher than that reported from developed countries. During the period of November 1991 to September 1992, Rotavirus was constantly detected. Between December 1991 and January 1992, positive rate was lower than subsequent months. In these months more than 25% of samples were positive for Rotavirus.

Conclusion are as follows:

1) Rotavirus is the most common cause of acute diarrhea in Zambian children.
2) Rotavirus is more common in younger age group.
3) Mortality rate of Rotavirus infection is high.
4) There is no seasonal variation in Rotavirus infection in Zambia.

This work was done as a part of the Infectious Disease Project, a technical cooperation between Japan and Zambia. It is supported by the Japan International Cooperation Agency (JICA).

33 PREVALENCE OF ENTERIC VIRUCES IN CHILDREN IN KARACHI, PAKISTAN

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To study the prevalence of enteric virai infections in children in Karachi, Pakistan.

Fecal samples were collected from patients of acute watery diarrhea aged ≦5 years in Civil Hospital Karachi. Feces from aged matched healthy children were studied as controls. HeLa, HEL, RD-18S cells were used for isolation of enteroviruses. Rotavirus and enteric adenovirus (EAd, Ad-40, 41) were detected by ELISA. Antibody against hepatitis A virus (HAV) was measured by ELISA.

During Oct. 1989-Sept. 1991, 322 diarrheal patients and 126 healthy children were studied. Enteroviruses were isolated from 144 (44.7%) diarrheal and 61 (48.4%) healthy children. Isolation positives were mainly infants and there was no seasonal cluster. Most of the isolated viruses were non-poliovirus and their serotype distributed widely.

Rotavirus were detected from 26.1% of diarrheal and 0.8% healthy children, primarilly from infants aged ≦1 year.

EAd positives were 0.2% in diarrheal and 0% in healthy children. Prevalence of HAV antibody was 82% in children in 3 years and 94% in 5 years of age.

Prevalence of enteric viral infections was prominently high among children in Karachi, especially those in ≦2 years of age.

34 ESTABLISHMENT OF STRATEGY FOR THE PREVENTION OF POST-TRANSFUSION HEPATITIS B IN KENYA

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KEMRI and JICA have been collaborating in research on hepatitis B for more than 5 years in Kenya. The production of HBsAg diagnostic reagent has been started and we could get the first reagent kit made in Kenya at the end of 1989. We investigated the circum stances of production of R-PHA cells and found that R-PHA cells can be produced in KEMRI derived from an antibody purified in Japan from an antiserum raised in horse. Although a comparable reagent to commercial ones could be produced in KEMRI using the horse antibody, use of horse as a host of immunization seems to oppose to our final goal to make a reagent with domestic materials. For the reason mentioned above, we had to change our objectives and intended to investigate the feasibility of use of antibodies raised in guinea pigs, rabbits and sheep which are available locally in Kenya. We could get good results from purified antibody raised in albino guinea pigs, because of the very high level of antibody from small and middle scales. These results showed that we could product R-PHA cells in large quantity with domestic materials. Furthermore the study of localy and mass production was under way and then we had enough to do to continuously supply government blood banks to screen donated blood. Therefore the workshop held at KEMRI was financed by JICA and coordinated and facilitated by both KEMRI and JICA scientist and technologist. Participating technologists and technicians, were drawn from 8 government blood banks. The major objectives of the workshop, were to teach these participants how to use the locally developed reagent and to re-emphasized the public health importance of hepatitis B. It is as a result of such research findings that strategy for the prevention of post-transfusion hepatitis B has established.

35 FOOD HABIT OF THE POISONOUS SNAKE YOUNG HABU, *TRIMERESRUS FLAVOVIRIDIS* ON THE AMAMI AND TOKUNOSHIMA ISLANDS

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Natural food habits of the poisnous snake young Habu, *Trimeresurus flavoviridis* on the Amami and Tokunoshima Islands in Japan were surveyed in 1991 to 1992. 458 males, 353 females in the Amami Islands and 536 males, 375 females in the Tokunoshima Island were examined.

Food of young Habu belong in Mammalia, Aves, Reptilia and Insect of 9 species. In the Amami Islands 72% cold-blooded animals as Reptile, Ryukyu little brown skink, *Lygosoma pelloplearum*, Ryukyu green grass lizard, *Takydromus smaragdinus* and Gecko, *Gekko* sp. mainly Ryukyu little brown skink, 26% as Rodents, Watase's shrew, *Crocidura horsfieldi watasei* and Amami spinous country-rat, *Tokudaia osimensis osimensis* and one case as Insect, cockroach, *Rhabdoblatta guttigera* were observed. On the other hand, in the Tokunoshima Island 78% warm-blooded animals as Rodents especially Watase's shrew, 21% as Reptile Ryukyu little brown skink, Ryukyu green grass lizard, Gecko and Ryukyu rice-paddy snake, *Natrix pryeri pryeri* and a bird, *Zosterops palpeblosa loochooensis*.

In the case of the Tokunoshima Island in winter season January to March 16 cases of Rodents and one case of Reptile were found. It's mean that the young Habu hunt prey in winter season too but in the Amami Islands only one case were found.

Different of species and number of prey in both Islands that depend on distribution and number of natural food inhabit in its.

36 THE REPRODUCTIVE CYCLE OF THE FEMALE HABU

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The reproductive cycle of the female Habu, *Trimeresurus flavoviridis* was studied. Vitellogenesis started in late April, and progressed in parallel with a striking increase of serum estradiol. The genital organs and the liver showed characteristic changes in the estrous period. Mating occurred in late April, too. Both ovulation and parturition happened early in June, when the level of estradiol fell abruptly. After parturition no sperms were found anywhere in the genital tract except for the infraepithelial alveolar glands of the transition region. Immunohistological study by anti-estradiol antibody suggested that estradiol is synthesized in the adrenals. In comparison with serum estradiol, the fluctuation in serum progesterone was within a small range. Serum progesterone rose slightly in spring, then after a slight decrease it rose again just before ovulation. During late autumn and winter the level of serum progesterone stayed low. FSH in hypophysis was recognized by immunohistological staining in October, although we could not detect any rise in serum FSH. LH in hypophysis was recognized from July to October, and serum LH rose in July. It is known that the injection of human FSH produces vitellogenesis in nonmammalian vertebrates. So vitellogenesis and other active changes in estrus need not only gonadotropin but also other factors, especially temperature for poikilothermic animals. The other pituitary hormones, GH, ACTH, and prolactin also exhibited interesting seasonal changes.

Our results show that the most suitable time for habu-hunting to reduce the danger of Habu-bite is before mating, from March to early April in Amami Islands.

37 AN EPIDEMIOLICAL STUDY ON THE SNAKEBITES IN GUANGXI ZHUANG AUTOMOUS REGION, CHINA IN 1990

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The data on 974 cases of snakebites in Guangxi during the year 1990 were collected. The species of venomous snakes and the rate of occurrence of their bites were *Naja atra* (27.4%), *Bungarus multicinctus* (8.4%), *B. fasciatus* (2.7%), *Ophiophagus hannah* (1.2%) *Trimeresurus stejnegeri* or *T. albolabris* (20.9%), *T. mucrosquamatus* (13.4%), *Deinagkistrodon acutus* (6.2%), *Vipera russelli* (5.5%), *Azemiops feae, T. jerdonii* and sea snakes (0.8%).

During warmer months from April to October, 88% of bites occurred. 18% of the total bites occurred in residences, 18% were on roads, 26% were in agricultural fields and 17% were in mountains. 56% of bites occurred during daylight hours from 6 a.m. to 6 p.m. mainly in fields and mountains, while bites in dark hours mainly occurred in residences and on roads. 94% of bites were widely distributed between the ages from teens to sixties, and bites in males were two and a half times as frequent as those in females. 21% of the total bites occurred during handling or catching the snakes, and the rate was very high in *N. atra* (50%) and *O. hannah* (92%). Fatal cases were 23 (2%) and higher fatality rate were by *O. hannah* (46%), *B. multicinctus* (10%) and *B. fasciatus* (8%). That of *N. atra* was rather low (0.7%). Most of the bites were treated by traditional Chinese herbal medicine.

38 EFFECTIVENESS OF PROTEASE INHIBITION IN SEVERE MAMUSHI BITE

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In Japan we often see Mamushi bite. It is estimated that Mamushi bite are 3,000 cases per year. Most of them are slightly or moderately cases, but rarely we can experience severe cases with DIC or multiple organ failure. Here we report 3 severe cases improved quickly after the early administration of protease inhibitor (FOY). First case was a 78 year old male. After Mamushi bite he had liver and renal disfunction, and low blood pressure. We started FOY 5 days after admission when we found sendency of DIC. He had acute heart failure and paralytic ileus as complications, but the course was good after administration of FOY. Second case was a 74 year old female. On admitted laboratory data was GOT 326, LDH 1,920, CPK 7,074, WBC 22,000, and hematuria. We administrated FOY one day after admission, and the course was good. Third case was a 44 year old male. The data on admission was GOT 71, LDH 417, CPK 2,224, WBC 18,900. In this case also the course after administration of FOY was good. Mamushi venom include several protease, which destroy blood or cause muscle necrosis. If we administer protease inhibitor (FOY) early, we can inhibit the action of the protease and also prevent DIC. Therefore we might expect effective treatment by protease inhibitor (FOY) in severe Mamushi bite.

39 THE BOX JELLYFISH (*CHIROPSALMUS QUADRIGATYS*) STINGS IN OKINAWA

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There has been no official report on the stings by the jellyfish in the Ryukyu Islands. But it has been well known to the general islanders that the stings are caused by several kinds of the jellyfish, of which Yamaguchi (1982) pointed out the box jellyfish, *Chiropsalmus quadrigatus* as chief assailants.

The following are the facts revealed by the investigations into 67 cases of the stings by *Chiropsalmus quadrigatus* from 1956 to 1991: —

The stings occurred from June to September, with August as a peak, showing 33 cases (50.8%). The stings took place within 5 meters' distance from the seashore, totalling a half (29 cases). The degrees of conditions were mostly slight for 46 cases (70.8%), medium for 11 cases (16.9%) and serious for 7 cases (10.8%). There was one death which occurred about one hour after the accident when a boy, age 13 was stung in the lower extremities of the body in August 1961.

The *Chiropsalmus quadrigatus* are distributed around the whole Okinawa Islands, including Okinawa Main Island, Miyako Island, Ishigaki Island and Iriomote Island.

When stung, people feel a keen burning pain, getting a welt on the skin touched by their tentacles. In serious cases, the skin develops necrosis and discoloration, leaving their traces for a long time. In some cases, conditions were so serious that the victims' heart and respiration failed. This means that in the case of an infant a sting may easilylead to very serious conditions.

When grown up, this box jellyfish become big, measuring 10 cm, with their tentacles reaching more than 50 cm. Being transparent in color, they are rarely recognized by the swimmers before they are stung.

40 INTEGRATED CONTROL OF JAPANESE ENCEPHALITIS VECTORS: —AN INQUIRY WITH MATHEMATICAL MODEL—

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A main vector of Japanese encephalitis (JE) in east and southeast Asia is a mosquito, *Culex tritaeniorhynchus*. Control of this species is quite difficult due to temporally and spatially heterogenous and vast larval habitats (rice fields and associated irrigation/drainage systems), high reproductive potential, wide flight range, exophily, and, in some localities, insecticide resistance acquired as a byproduct of agricultural chemical application. To explore the possibility for reducing human risks to be infected with JE virus, a JE transmission dynamics model involving swine (virus amplifying host), humans and a vector mosquito was developed. In this model system, vector abundance is influenced by availability of bloodmeal hosts, either swine or humans as well as larval survivorship which includes both density-independent and density-dependent components. Human risks of JE virus infection can be evaluated with infective mosquito density multiplied by the probability of biting humans. Model consequences suggest; (1) Integration of multiple measures can substantially reduce human risks, even if their effectiveness is solely not sufficient, (2) Effects of each measure may overlap with that of others, (3) Combination of mutually independent (without overlapping effects) measures does not lower cost-effectiveness thus is preferred, (4) Relative effectiveness of measures changes with the initial conditions, therefore baseline data for each locality is essential for adoption of measures being more effective in that locality, (5) with regard to measures against adult vectors, relative numbers and attractiveness among humans, swine (and also other domestic bloodmeal hosts such as cattle if exist) are especially important factors influencing the relative effectiveness of each measure, (6) Larval mortality from a indigenous predator complex is a hidden factor influencing the level of human risks, and (7) Changes in predation mortality within 10% may still enough to cause substantial change in the level of human risks. Though it would be difficult to exterminate virus amplification among swine by reducing the basic reproductive rate below unity, it appears possible to reduce human risks substantially by a combination of interventions appropriate and acceptable to each area. As a basic strategy for integration of multiple measures against mosquito vectors with wide flight ranges, a concept of "horizontal integration" is proposed.

41 A ROLE OF MIDGUT IN THE SUSCEPTIBILITY OF MOSQUITOES TO CHIKUNGUNYA VIRUS

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Chikungunya virus concentrations in the head/thorax, midgut and other abdominal parts were measured to compare virus multiplication in the mosquito *Culex tritaeniorhynchus, Aedes aegypti* and two strains of *Ae. albopictus* (Hyogo and Oahu strain) by plaque assay with BHK 21 cells. In *Cx. tritaeniorhynchus*, CHIK virus disappeared from the midgut by the 2nd days after feeding. In *Ae. aegypti*, CHIK virus was observed in 12% of the midgut for 2 weeks after infection. In the Hyogo strain of *Ae. albopictus*, CHIK virus was observed in 60% of the midgut for 2 weeks. CHIK virus was not detected in the other tissues on the 4th day after feeding. In the Oahu strain of *Ae. albopictus*, all the midgut specimens were positive for CHIK virus by the 7th day after infection. On the 7th and the 14th day after infection, CHIK virus was detected in the other parts than the midgut. For these experiments, a role of midgut in the susceptibility of mosquitoes for CHIK virus was thought to be important. It was discussed the factors for the susceptibilty in the midgut were digestive juices and peritrophic membrane.

42 HOST PREFERENCE OF *ANOPHELES MINIMUS*

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The host preference of vector mosquito is one of the important parameters determining the relative importance of vector mosquitoes in disease transmission, though it is not easy to quantify the degree of their preference to man. In the classic method examining host preference of vectors, two kinds of animal baits were exposed to mosquito feeding and compared the number of attracted mosquitoes to the baits. This method gives us an information about the host preference but a new analytical method have to be developed to quantify the host preference through the comparison of the number of attracted mosquitoes between the baits. A new index of host preference, Hp, was defined by the following equation as the probability of mosquitoes feeding on one kind of animals when two kinds of animals are available for blood feeding;
$Hp = B\,Mh/(H\,Mb + B\,Mh)$ where B and H are the number of animals used in the bait collection and Mb and Mh are the number of mosquitoes attracted to the two kinds of animal bait.

Using the index, host preference of *Anopheles minimus* in Northern Thailand and Ishigaki-jima populations were compared based on the results of bait collection derived from Chiangmai in November, 1990 and Ishigaki-jima in July, 1992. Because of the geographic isolation and different history of mosquito control in these two areas, Ishigaki-jima population was expected to show lower preference to man than Northern Thailand population. The result of our analysis showed that the lower preference to man in Ishigaki-jima population will be expected only when the following inequality holds; $kw/k > 40$, where k and kw are the efficiency of sweeping collection and double net collection, respectively.

43 OCCURENCE OF VECTOR MOSQUITOES OF JAPANESE ENCEPHALITIS IN NORTHERN THAILAND IN RELATION TO THE RICE CULTIVATION

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As a part of the epidemiological study on Japanese encephalitis in Thailand, we conducted a monthly quantitative field survey on the vector ecology and habitat conditions of the vectors. A variety of rice fields, which were the main habitat for vector larvae, was examined on the density and the species composition of larvae, the prevalence of water, the water quality and the height of rice or grasses.

Among six species which were reported as the vectors, three species, *Culex tritaeniorhynchus, Cx. vishunui* and *Cx. gelidus* were incriminated. The other three species, *Cx. whitmorei, Cx. pseudovishunui* and *Cx. fuscocephala*, were not often encountered in our study fields. *Cx. vishunui* may have been underestimated its role for transmission of the disease because the species is hardly distinguished from *Cx. tritaeniorhynchus* by adult specimens, while *Cx. vishunui* was so common that it was the most predominant in some rice fields in our survey tergetted to larvae which were easily identified the species.

The larval density was apparently high in the two cropping rice fields, and the initiation time of increase nearly coincided to ploughing for the next transplanting. On the other hand in rice fields of the single cropping, the density was lower than the two cropping areas. The seasonal prevalence of adult populations examined at

pigsties showed a similar pattern to that of larval populations when the pigsties were involved in the same rice field as the larval populations. It was confirmed that the practice in rice culturing inevitably affected the occurrence of the vectors.

44 EFEECT OF THERMAL ACCLIMINATION ON BLOOD PRESSURE AND GROWTH IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) Report II

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Effect of thermal acclimation on body weight (BW) and blood pressure (BP) was studied in SHR and Wistar-Kyoto rats (WKY). From the birth time (Type A) or the age of one month old (Type B), male SHR and WKY were chronically exposed to cold (10°C), hot (30°C) and thermoneutral conditions (22°C, control) under 12 hr dark/light cycle with food and water *ad libitum*. Three or four rats were kept in a plastic cage. Systolic BP was measured by tail cuff method.

In type A of thermal acclimation of WKY, values (Mean±S.E.) of BP and BW were 134±3 mmHg and 310±7 g in cold-acclimated WKY (WKY-C, N=6, 3.9 months old M), and 117±4 mmHg and 234±3 g in heat-acclimated WKY (WKY-H, N=7, 4.1 M), respectively. And in Type B of WKY, values of BP and BW were as follows: 143±2 mmHg and 349±6 g in WKY-C (N=5, 4.6 M); 115±5 mmHg and 314±7 g in WKY-H (N=8, 4.3 M); 127±4 mmHg and 324±15 g in control rats exposed to thermoneutral condition (WKY-N, N=6, 4.0 M), respectively. Values of BP in Type A and B of WKY-C were significantly higher ($p<0.05$) than those in WKY-H. In WKY, there was no significant difference between BP values of Type A and B. In type A of SHR, values of BP and BW were 179±5 mmHg and 318±7 g in SHR-C (N=7, 5.1 M), and 180±3 mmHg and 222±4 g in SHR-H (N=4, 4.1 M), respectively. In Type B of SHR, values of BP and BW were as follows: 184±4 mmHg and 344±7 g in SHR-C (N=10, 4.4 M); 176±5 mmHg and 264±17 g in SHR-H (N=9, 3.9 M); 170±5 mmHg and 308±11 g in control (N=8, 4.2 M), respectively. There was no significant difference between BP values of SHR-C and SHR-H. Values of BW in cold-acclimated rats were sigificantly ($p<0.01$) greater than those in heat-acclimated ones in Type A and B of SHR and in Type A of WKY. And in SHR-C, WKY-C and WKY-H, BW values of Type A were significantly smaller ($p<0.01$) than those of Type B. The development of cardiovascular control system of SHR is susceptible to preweanling environmental influences, *i.e.* a repeated handling (3) and a rearing by normotensive mother (2). In this study, BP values were not reduced by heat-acclimation in both Type A and Type B of SHR, though a mild hypertension was induced by cold exposure in normotensive WKY (1). On the other hand, in SHR and WKY, values of BW in heat-acclimated rats were smaller than those of cold-exposed rats. It remains to be solved why the development of hypertension in SHR is resistant to the environmental influences.

45 INHIBITION OF IL-1 SERECTION AND PRORIFICATION OF MACROPHAGE DUE TO HEAT LOAD

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The macrophage is activated by LPS in the blood to induce synthesis of endogenous pyrogen and releases it

into the blood, and fever is caused. Interleukin-1 (IL-1) is the principal substance among endogenous pyrogens, which causes varient physiological regulations including a fever response. IL-1 stimulates organum vasculosum laminae terminalis (OVLT), which exists on the side wall of third ventricle in the brain and causes the release of prostaglandin E_2 (PGE_2) there. PGE_2, a cascade of arachidonic acid, reaches to preoptic area and anterior hypothalamus (PO/AH), causes a reset of the setpoint, and thus triggers a fever response. The reaction as follows is important during bacterial infection: LPS -macrophage-IL-1-PGE_2-fever. One might wonder why even extremely high fevers seldom exceed 42°C. It is postulated that there is a self-limiting mechanism, such as a negative feedback loop. On the other hand, the heat shock protein (hsp 70) can be induced by heat stress. However, hsp 70 relates to a febrile response or not has been seldom discussed. The present study, therefore, investigated influences of heat shock on macrophage proliferation and IL-1 secretion, studied the induction hsp 70 in macrophage, and examined the correlation between them. RAW 246.7 cell and U-237 cell were used and cultured with RPMI 1640 medium supplemented with 10% FBS. The result of methyl-[^{3}H]-thymidine incorporation in RAW 246.7 cells showed that the proliferation of the macrophage was suppressed by a heat load of 39°C for 2 hr. Enzyme -linked immunosorbent assay (ELISA) showed that the activity of IL-1 secretion induced by LPS was also suppressed by heat load. Suppression of activities of the macrophage including proliferation and secretion suggent that negative feed back loops exist where the end product, heat, may act back on the macrophage. The induction of heat shock protein (hsp 70) by heat shock in the macrophage was detected by Western-blot and Northern-blot analysis. In U-937 cells, the increase of hsp 70 band appears to be related to the extent of inhibition of IL-1 secretion. The physiological significance of induction of hsp 70 in macrophage are assumed as follows: 1) To prevent heat denature of pre -rebosome from heat shock during heat stress 2) To suppress the biosynthesis and secretion of IL-1 by mean of combination of heat shock transciption factor (HSTF) to IL-1 gene on the chromosome, thus, a negative feedback loop is formed and result in a subsiding of a fever.

46 ANALYSIS OF MICRO-ENVIRONMENT ON SWEAT SKIN COVERED BY THERMAL SWEATING OF THE SKIN COVERED BY CLOTHING MATERIAL

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In order to clarify the effect of different clothing material on the micro-environment between clothes and skin, analysis of thermal sweating was investigated by using the sweat capsule method. Changes in the micro-environment induced by sweat capsules, clothes and skin sweat, were detected by hygrometer cambined with a computer and DC recorder. Moisture changes in the micro-environment of the sweat capsule may depend on the following three factors such an absorption, penetration and evaporation of wet clothing material on the sweating skin. Four sweat capsules connected to dry N_2 Gas (1 liter/min, capsule) were fixed on the anterior chest, each covered by a small piece of different kinds of clothing material such as silk, cotton and nylon. 24 experiments using 6 male subjects were performed. After a 10-20 min rest in an environmental control room (27°C, 60% rh), subjects were submitted to a 43°C water bath heat load to bilateral lower extremities for 30 min.

Sweat volume and initial increase-rate of thermal sweat in the medial portion of the chest due to 43°C water bath heat load were significantly higher than the lateral portion of the chest. There were no significant changes among the three different kinds of clothing material on sweat volume, nor were there any effects of a different setting mode in the order of clothing material on sweat volume. Sweat onset time for each clothing material was in the order: nylon>silk, and cotton>silk. This may be attributed to the difference in absorption and penetration among each clothing material.

Recovery time of sweat-curve after the cessation of heat load was significantly longer in the medial portion of the chest than the lateral portion of the chest, which

could depend on differences in sweat volume in these portions of the chest. Recovery time, however, was significantly different kinds of among three different clothing material and the results obtained was in the order of: cotton>silk>nylon. In the present study, analysis of moisture changes caused by clothing material was analyzed at the level of micro-environment, and the results obtained will be useful for analyzing the best mode of daily wear that is optional for human an body physiology.

47 STUDY ON MECHANISMS OF HEAT ACCLIMATIZATION DUE TO SWEATING

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Recently, most theories consider that central and peripheral inputs jn thermoregulation interact at a neural level in the hypothalamus of which effector mechanism indicates little or no increase in heat acclimated animals (habituation phenomenon). In this study, therefore, to clarify the mechanisms of heat acclimatization of permanent residents in tropical climate, heat tolerance and sweat response to heat load of tropical subjects in Chiang Mai and temperate subjects in Nagasaki were compared under identical conditions.

Male students in Chiang Mai (n=10) and in Nagasaki (n=10) volunteered in this study. Mean annual air temperature is reported to be 16.6°C in Nagasaki (32°44′N, 129°52′E) and 25.9°C in Chiang Mai (18°47′N, 98°59′E). The Thai subjects were a little shorter and slightly leaner than the Japanese. Heat load was applied on the legs by immersing into hot water (43°C) for 30 min in the room at 26.6°C and 33% rh. Sublingual (oral) temperature was measured with a thermistor and local sweat rate was measured with a capacitance hygrometer-sweat capsule method. Change in oral temperature, sweat onset time and local sweat volume were compared between Japanese and Thai.

Initial oral temperature (36.76±0.11°C in Japanese, 36.71±0.23°C in Thai) was identical, and no sweat was observed before heat load. Mean sweat onset time (9.3±2.1 min chest in Japanese, 16.6±5.6 min chest in Thai) was significantly longer and local sweat volume (10.19±5.00 mg/cm², chest in Japanese, 1.39±0.91 mg/cm², chest in Thai) was significantly smaller in Thai subjects than Japanese, however, oral temperature (37.18±0.32°C) of Thai subjects was kept slightly lower than oral temperature (37.42°C±0.10°C) of Japanese even in 30 min heat load. Sweat volume on the abdomen was larger than that on the chest in 9 of 10 Thai subjects. On the contrary, sweat volume on the chest was larger than that on the abdomen in 7 of 10 Japanese subjects. These results suggest that heat tolerance of tropical subjects is due to a more efficient evaporative the greater heat loss brought about by their long term exposure to heat.

48 COLD TOLERANCE OF HYPOTHERMIC SYRIAN HAMSTER DUE TO ACUTE COLD EXPOSURE

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Syrian hamster is a hibernator which is possible to live in a low core temperature at 5-8°C during a hiberna tion in winter. Therefore, this study experimented to clarify an ability of cold tolerance in the stage of non-hibernation. Syrian hamsters were submitted to the acute cold exposure at 0°C and the results obtained was compared with those of Wistar rat. Temperatures in the abdominal cavity (Tab), abdominal skin (Tabs),

back skin (Tbs) and brown adipose tissue (Tbat) were measured with a thermistor thermometer every minute. Respiratory rate and ECG also were recorded with a data recorder.

Tab of Syrian hamster decreased quickly compared with Tab of Wistar rat during the cold exposure, and the minimum Tab (6.82°C) in Syrian hamster was lower than 15.14°C in Wistar rat. Tab of Syrian hamster increased just after the stop of cold exposure and Syrian hamster survived, but Wistar rat could not recover though Tab of Wistar rat slowly increased to 19.78°C.

The insulation of back skin is better than that of abdominal skin in both animals as a result of the differences of Tabs and Tbs from the temperature of cold exposure, and the insulation of the skin in Wistar rat was superior to that in Syrian hamster.

Respiratory pattern changed eupnea to bradypnea during hypothermia in both animals. Heart rate of Syrian hamster changed normal range to bradycardia characterized by long interval time and ECG pattern changed to high amplitude with decreasing of Tab. In rat, ECG pattern of high amplitude and low frequency appeared again after ECG temporally disappeared at 26.45°C of Tab. But the disappearance of ECG was not observed in Syrian hamster.

The difference of the back skin and the abdominal skin insulations suggests that the posture to curl itself up in cold environment is useful for a protection against a heat loss from body surface. The insulation of skin in Wistar rat was better than that in Syrian hamster. It was well reported that the metabolism of Wistar rat was higher than that of Syrian hamster in summer. Though Wistar rat is powerful compared with Syrian hamster from views of the insulation and the heat production, the ability of cold tolerance in Syrian hamster was superior to that of Wistar rat during hypothermia. Syrian hamster of hibernator possesses high ability of cold tolerance in hypothermia not only in hibernation but also in arousal stage.

49 A SURVEY OF INTESTINAL PARASITIC INFECTION IN KOK TRAP COMMUNE, CAMBODIA; USING THE AGAR PLATE METHOD

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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, not only for its reliability but also for its field applicability in developing coutries based on results of our previous studies in Chiang Mai Province, Thailand. Moreover, we reported previously that this method was revealed to be sensitive enough to detect *Stongyloides* even when only one worm was present in the fecal sample. Since prevalence rate of *Strongyloides* among villagers in Huey Keaw Village, Chiang Mai Province was 34%, 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, stool examinations including the agar plate method for villagers in Kok Trap Commune, Kandal Stung District, Cambodia was performed to determine the present state of parasitic infections in the village.

The positive rate of intestinal parasites seen among 100 villagers was 82%. The most common parasite was hookworm (71%) followed by *Strongyloides* (19%), *Ascaris lumbricoides* (3%), *Trichuris trichiura* (2%), *Giardia lamblia* (2%), *Entoamoeba coli* (1%) and *Enterobius vermicularis* (1%). Out of 71 cases of hookworm-positive, *Necator americanus* was found in 11 cases, *Ancylostoma duodenale* in 16, and one was mixed-infected.

Cambodia has been reported to be sufferd from war. We can rarely find the data about epidemiology or health situation in Cambodia. It had been presumed that people possess many kinds of intestinal parasites, but the results showed the parasites except hookworm and *Strongyloides* is not so prevalent unexpectedly.

50 AN EPIDEMIOLOGICAL STUDY ON *STRONGYLOIDES* INFECTION ON MIYAKO ISLAND OF OKINAWA PREFECTURE, LOOKING FOR ANY POSSIBLE RELATION BETWEEN INFECTION AND FARMING ACTIVITY

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We have been carrying out surveys into the prevalence of *Strongyloides* infection in almost all regions of Okinawa since 1988, and have previously reported that almost all *Strongyloides* carriers are over the age of 40 and that there is a marked regional difference in prevalence. A preliminary questionnaire survey was conducted on Miyako Island, into infection risk factors.

The questions were whether people had ever engaged in farming or not, whether they had ever used human manure and whether or not they went barefoot in their fields. The proportion of infected cases found for each behavioral factor is shown for both sexes. From these results, it seems probable that engagement in agriculture itself was not a major risk factor for *Strongyloides* infection on Miyako Island. Further study along different lines is indicated to determine the relevant factors in the spread of this infection.

51 EFFECTS OF FLUBENDAZOLE AND MEBENDAZOLE AT A LOW DOSE ON THE GROWTH OF *ANGIOSTRONGYLUS CANTONENSIS* IN RATS

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Many human cases infected with *Angiostrongylus cantonensis* have been found in Taiwan, Thailand and the Pacific islands with a small number of cases in some parts of tropical, subtropical or temperate zones including Cuba and Ivory Coast (Hung and Chen, 1988). Despite the infection, no suitable chemotherapy has hitherto been established. Though benzimidazoles such as mebendazole, flubendazole and albendazole are promising in killing *A. cantonensis* in human cases, there still remains a problem to overcome: any adverse effects for which the worms drastically killed might be responsible (Maki and Kanda, 1992). The present communication reports effects of flubendazole and mebendazole at a low dose on the growth of this nematode in rats.

These benzimidazoles were each administered at 10 mg/kg to rats harbouring the developing larvae 3 or 10 days post-infection, or to rats habouring the adult worms 10 weeks post-infection. All the rats were autopsied 8-10 weeks after the medication. Almost no worms were recovered from the rats medicated 3 days post-infection. The larvicidal effects of the drugs administered 10 days post-infection were not so high as those 3 days post-infection. However, the larvae still surviving in the rats medicated 10 days post-infection were found to have been inhibited significantly from their growth in length, width and weight except the length of the larvae in the rats given mebendazole. An inhibition from their growth was also demonstrated with the observation that no first-stage larvae (L1) were released in the faecal pellets from the rats medicated 10 days post-infection and examined just prior to the autopsy, at which L1 were released from non-medicated rats. On the other hand, when the drugs were administered 10 weeks post-infection, no effects of the drugs were seen in the number, body size and weight of recovered worms and L1 release. It was reported that a

higher dosage in total was needed to suppress L1 release. Flubendazole at 10 mg/kg/day for 3 consecutive days reduced drastically the number of L1 released in the faecal pellets about 6 days after the last dosing (Maki, 1991). It was confirmed in the present study that the developing larvae are more sensitive to the drugs than the adult worms. The symptoms in human cases are manifest usually at least one week after this nematode infection (Punyagupta *et al.*, 1970). Flubendazole and mebendazole seem to be useful for the inhibition in the growth of this nematode unless the drugs are administered too late.

52 ANTICESTODE EFFECTS OF GASTROGRAFIN

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Cestodiasis is now an important parasitic disease in Japan, having been imported from the developing countries. Gastrografin is often used clinically as a vermifuge. The treatment is simple, and cestodes are living when they are excreted. Therefore, this drug is recommended for use in studies of the morphology, physiology, and immunochemical characteristics of cestodes. The anticestode mechanism of the drug is not known. We studied the effect of the three main constituents of Gastrografin on cestodes by external cultivation of a *Taenia saginata* excreted in its entirety by a female patient. Gravid or mature proglottides were washed with a mixture of RPMI-1640 medium, penicillin (400-500 units/m*l*), and amphotericin B (30 μg/m*l*), and then incubated in one of three media, supplemented with NaOH, meglumine, or amidotrizoic acid. Results were compared with those with 0.85% NaCl, Gastrografin, RPMI-1640, or fetal calf serum, the four controls. Five proglottides were grown in each medium at 37°C in a CO_2 incubator. Every 24 hr, the response by movement to being touched with a syringe was observed. Survival in the different media was also recorded for each individual, and maximum survival time for each group was compared. The media containing NaOH or meglumine shortened survival. Each proglottid was examined pathohistologically. Pathological changes were found in the tegmentum and subtegumental structures and in the number and distribution of calcareous bodies in the medium with NaOH. The anticestode effects of Gastrografin seem to arise from the constituents NaOH and meglumine as well as from the high osmolarity and increased intestinal peristalsis.

53 ANTI-PHEX ANTIBODY, AS A SCREENING METHOD FOR SUBCLINICAL AMEBIASI

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In our previous study, we have demonstrated that there is a significant difference in the antibody responses of the sera isolated from various stages of amebiasis patients on enzyme-linked immunosorbent assay (ELISA) using threetypes of *Entamoeba histolytica* antigens. The sera isolated from acute-phase amebiasis revealed a high titer of anti-PHEX and a low titer of anti-CRAR IgG responses. Conversely, the sera isolated from chronic amebiasis revealed a high titer of anti-CRAR and a low titer of anti-PHEX IgG responses. Therefore, to evaluate a utility of these ELISA methods with three types of antigens as a screening test for the inhabitants in endemic areas for amebiasis, we applied these ELISA to the sera isolated from Indo-China refu-

gees with subclinical amebiasis.

Seventy sera isolated from Indo-China refugees, who were positive for *E. histolytica* trophozoites or cysts in their stools, and fourty sera isolated from Japanese healthy subjects, who were free from parasitic infections and abnormal findings by periodic health check up, were examined by serological tests including ELISA and Western blotting. The cut-off values of these three ELISA methods were calculated by the mean absorbance values of the sera from Japanese healthy subjects. Approximately 70% of the sera from the refugees revealed positive reaction on ELISA with the crude extracted antigen. The positive rates of these sera on ELISA with the CRAR and PHEX antigens were 50% and 30%, respectively. As also shown in our previous study, there was no correlation between the ELISA titer using CRAR and PHEX antigens. A low molecular weight substance has been identified as a major antigen by Western blotting analysis of the PHEX antigen. Virtually all sera from the Indo-China refugees reacted with such a molecule on Western blotting with PHEX antigen though majority of these sera did not show positive reaction on ELISA with the PHEX antigen. All sera except one isolated from the refugees, who discharged pathogenic amoebae, were positive by ELISA with the PHEX antigen. However, thirteen out of 15 sera from the refugees, who discharged non-pathogenic amoebae, showed negative on ELISA with the PHEX antigen.

These findings probably suggest that improved ELISA with the PHEX antigen may be an efficient tool for screening the asymptomatic subjects who are infect ed with pathogenic amoebae in endemic as well as non-endemic areas.

54 IMMUNODIAGNOSIS OF HUMAN SYSTICERCOSIS II. RESULTS OF THE IMMEDIATE TYPE OF SKIN TEST REACTION

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Human cysticercosis is endemic in the northeast area of China, although its mechanism of infection and epidemiologic status have not been clear yet. We have planned to make a epidemiologic survey for human cysticercosis in an endemic area of Kirin Province, China. Prior to make the survey, suitable immunodiagnostic systems for the screening of cysticercosis patients were examined. In this study we report our initial attempts to evaluate a immediate type of skin test for the one of tools using with screening of patients. Extract from the scolex with cyst wall for the skin test and cyst fluid for the ELISA system obsained from the muscle of pigs naturally infected with cysticerci were used as the antigen. The skin test was carried out to 54 hospitalized cysticercosis patients defined with CT scan at the Military Hospital in Kirin Province. The case showing 9 mm or more in wheal diameter and/or 20 mm or more in erythema was regarded as the positive. ELISA was also performed in microtiter trays to 33 sera of 54 hospitalized patients together with 13 control sera of Japanese. Setting lowest positive limit at greater than 2 standard deviations above the mean of control sera we established the minimum OD value required for a positive titer. As the result of skin test 38 (86.4%) out of the 44 cases of cerebral cysticerei infection and 8 (88.9%) out of the 9 cases of muscle cysticerci infection showed positive reaction. On the other hand 17 (68.0%) out of the 25 cerebral cases and 7 (87.5%) of the 8 muscle cases were positive by ELISA. The mean OD value by ELISA obtained from cerebral cysticercosis patients sera was lower than that from muscle cysticercosis patients sera but no significant difference was observed.

55 COLLAGEN SYNTHETIC ACTIVITY IN THE FIBROTIC LIVER OF *SCHISTOSOMA MANSONI*-INFECTED MICE

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To clarify the mechanism of liver fibrosis in schistosomiasis mansoni, we conducted some biochemical investigations using experimental murine model infected with *Schistosoma mansoni*. This communication deals with identification of the major site of collagen production in the fibrotic liver and of some soluble factor(s) which is responsible for the increased collagen production in the fibrotic liver. Fibrotic liver prepared from *S. mansoni*-infected mice for 9 weeks contained 15-times more collagen than normal. Hepatic collagen content increased rapidly during the 50 to 80th days of infection, and then increased much more slowly. Collagen fibers increased in the fibrotic liver primarily localized in the egg granulomas. The collagen production, as measured by the formation of labelled protein-bound hydroxyproline, in the liver slices prepared from infected mice increased 8-times more than that of uninfected controls. However, the collagen production of the isolated egg granulomas was much lower than that of infected liver. Such a high level of hepatic collagen production in the liver slices was first demonstrated 49 days after infection and observed until 100 days after infection. Intraperitoneal injection of the crude extract of egg granulomas to normal mice had no effect on their activity of hepatic collagen synthesis. In contrast, a significant increase in the collagen production was demonstrated in the liver of mice treated with the crude liver extract prepared from infected mice. However, the addition of such a liver extract has no effect on the collagen production by primary cultured hepatocytes. On the other hand, the collagen production by primary cultured fibroblasts was significantly stimulated by pre-incubation with TGF-beta or the supernatant fluid of *in vitro* culture of egg granulomas.

These findings probably suggest that liver cells may play a major role in the increased collagen synthesis in the livers of mice infected with *S. mansoni*. Moreover, some soluble factor(s) is probably responsible for the increased hepatic collagen production in murine schistosomiasis mansoni.

56 NECROSIS OF PRIMARY HEPATOCYTES AROUND *SCHISTOSOMA JAPONICUM* EGGS *IN VITRO*

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The main pathophysiological changes in schistosomiasis japonica and mansoni are induced with inflammatory reactions around the eggs, which were deposited in portal and mesenteric veins, and the secondary responses than the direct damages which were induced by adult worms themselves. It is well known that the immunological responses, especially cell mediated immunity, are concerned with the granuloma formation around the deposited eggs into portal and mesenteric vein. In experimental murine model, the liver necrosis and inflammatory responses around eggs were investigated at first. However, there are many unsolved questions about mechanisms of granuloma formation around schistosome eggs. There may be many possibilities about the causes and mechanisms of host-parasite relationship. The secretory substances of mature miracidium may induce granuloma reactions as host's defense mechanism.

In this preliminary experiment, the primary hepatocytes were cultured with the matured schistosome eggs *in vitro*. The morphological changes of hepatocytes around the eggs were investigated. The female worms, which were collected from the portal vein of the schistosome-infected mice at 8 weeks after

infection, were cultured for 36 hr *in vitro*. The produced eggs were cultured in RPMI 1640 medium including 10% inactivated FCS and antibiotics for 7 days. The primary hepatocytes were collected from a rat's liver, which was perfused with MEM including collagenase IV. The small pieces of liver, which was removed from body and cut with a scalpel, were passed through sterilized gauze and steel mesh. The cell suspension was centrifuged and other cells including Kupffer cells were removed. The hepatocytes and schistosome eggs were co-cultured in plastic dishes coated with collagen. The medium including insulin, glucagon, epidermal growth factor, dexamethasone, apurocinin and antibiotics was exchanged each other days. The degeneration of hepatocytes around schistosome eggs began from 4 days, but no changes of other hepatocytes. This result showed some substances, which were secreted from miracidium, might have cytotoxicity to hepatocytes and that the liver necrosis around schistosome eggs might be first pathophysiological change, not but immunological responses.

After this I will investigate the biochemical characteristics about the secretory substances of schistosome eggs.

57 RELATION BETWEEN ANNUAL RAINFALL AND NUMBER OF *BULINUS GLOBOSUS*, HOST SNAIL OF *SCHISTOSOMA HAEMATOBIUM*, IN KWALE, KENYA

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Snail surveys for schistosomiasis haematobia were carried out twice each month at small rivers in Mwachinga and Mtsangatamu villages in Kwale, Kenya for 10 and 6 years, respectively. Thereupon, the relation between annual rainfall and number of *Bulinus globosus* were studied. Rainfall in this area generally followed the typical bimodal pattern of the Kenyan coast, and had a major peak during April to May and a small one in November. Kadingo River which flowed into Mwachinga Village dried up in the dry season. The streams in Mtsangatamu Village flowed continuously throughout dry seasons.

The annual number of collected snails in Kadingo River varied from 964 to 4,031 (av. 2,428) and the annual rainfall varied from 440 to 780 mm (av. 580 mm). In some streams in Mtsangatamu Village, the annual number of collected snails varied from 257 to 1,483 (av. 839) and the annual rainfall varied from 496 to 914 mm (av. 728 mm). Snail numbers peaked generally in October to November and in January (before and after the short seasonal rains) in Kadingo River and peaked in March to April (beginning of the main rains) at the streams in Mtsangatamu Village. There is no clear relation between the annual rainfall and the number of collected snails in the rivers of both villages. In Kadingo River, the more rainfall during seven months (June-December) after the main rains was, the more the number of snails were. However, the annual number of collected snails suppressed at the low level when the annual rainfall in the preceding year was small (under 500 mm) and/or total amount of the rainfall in former year except the main rains was less than 200 mm. In the streams in Mtsangatamu Village, the annual number of collected snails was at a low level when the rainfall after the main one, consequently, the large number of snails were collected in case of small rainfall after the main one.

58 ASSOCIATION OF *OPISTHORCHIS VIVERRINI* AND HEPATOBILIARY DISEASES IN NORTHEAST THAILAND

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Epidemiological studies on *Opisthorchis viverrini* infection in rural communities in Khon Kaen, Northeast Thailand have shown the high prevalence and intensity of the fluke infection. The local people are infected by eating raw fresh-water fish (Koi pla) which contain metacercariae of the fluke. The high incidence of cholangiocarcinoma (CHCA) in the area has also been noticed. In this study, we measured IgG antibody titers to *O. viverrini* antigens by ELISA in patients with hepatobiliary diseases to investigate association between the fluke infection and the disorders in the area. The titers were indicated by absorbance value.

Patients with cholangiocarcinoma (CHCA) showed significantly higher positive rate of the fluke infection (89.4%) and antibody titers (mean=0.35) than patients with calculous cholecystitis (CCLT; 69.0%, mean titer=0.13) and victims of traffic accident (VTA; 42.9%, mean titer=0.10). No significant difference on the rate and the titer was observed between CCLT and VTA. Among the CHCA cases, the positive rate and the antibody titers of females with intrahepatic CHCA were lower than those of males.

Those observations showed strong association between high antibody titers to *O. viverrini* antigens and genesis of cholangiocarcinoma. Synergistic effects of other factors such as intake of N-nitroso compounds contaminated food should also be considered to explain the genesis of tumours especially in patients with low antibody titers, which will reflect light infection of the flukes, as was suggested by Thamavit *et al.* (1987). They showed that even a few *O. viverrini* infection cause the genesis of CHCA in hamsters administered with subcarcinogenic dose of a N-nitroso compound.

59 LEISHMANIASIS AND ITS TRANSMISSION MODE IN ECUADOR

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Cutaneous leishmaniasis is a major public health problem in the rural area of Ecuador. In the country the first case of the disease was published in 1920 more than 70 years ago. Since this period there have been various clinical cases published in distinct regions of Ecuador. We have carried out epidemiological studies on the disease in the country since 1982 and disclosed several characteristics of the disease, such as geographical distribution, vector transmission, reservoir hosts and cinical forms. In addition, many details have been clarified in reference to the species of the genus *Leishmania*, besed on the isolates from different endemic areas of leishmaniasis in the country. Accordig to the results obtained to date, there are several types of leishmaniasis found in the endemic area corresponding to the Pacific coastal region: *Leishmania panamensis, L. guyanensis, L. amazonensis* and *L. mexicana*. In the region of the Amazon *L. braziliensis* has been described and in the Andean highland *L. mexicana and L. major*-like parasites were found. Moreover, we recently described a new species, *L. equatorensis* sp. n., isolated from arboreal mammals living in an area endemic for

human leishmaniasis. Therefore, in total there are seven species of parasitic agents which are thought to be classified thus far (Mimori *et al.*, 1989; Armijos *et al.*, 1990; Hashiguchi *et al.*, 1990, 1991).

With respect to the sand flies, we studied 15 endemic areas in nine different provinces. In total more than 40 species were found, from which at least 11 were newly recorded in Ecuador. If we add to these species the others which have already been reported, we have a total of 56 species, of which 16 are anthropophilic and thus must study their behavior as vector agents of leishmaniasis. To date, three species, *Lutzomyia trapidoi, L. hartmanni* and *L. gomezi* from the Pacific coastal lowland, and one species, *L. ayacuchensis* from the Andean highland, were recorded as probable vectors of leishmaniasis in Ecuador. As to the reservoir hosts, *Leishmania* strains were isolated from four mammalian species, *Choloepus hoffmani didactylus, Sciurus granatensis, S. vulgaris, Potos flavus* and *Tamandua tetradactyla* from the coastal regions, and from two, *Rattus rattus* and *Canis familiaris* from the Andean regions. Most of the isolates from humans, sand flies and mammalians were already identified by molecular characterization, such as isoenzyme electrophoresis, monoclonal antibodies and indirect radioimmune assay and schizodeme analysis of kinetoplast(k)-DNA as mentioned above, but some are at present being characterized (Co-workers: Drs. Eduardo A. Gomez L., Juan J. Alava P., Vicenta V. Coronel, Jose Rumbea G. and Hugo Jurado S., Ecuador, and Ken Katakura, Yoshitsugu Masumoto, Masato Kawabata, Hiroyuki Takaoka and Atsushi Hosokawa, Japan).

60 TRIALS OF LOW AND HIGH CONCENTRATIONS OF TOPICAL PAROMOMYCIN OINTMENT FOR CUTANEOUS LEISHMANIASIS

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There are many forms of treatments for cutaneous leishmaniasis. Recently, topical chemotherapy for cutaneous leishmaniasis has been reported, using paromomycin ointment in combination with methylbenzethonium chloride. In Ecuador, there is a report on topical treatment for cutaneous leishmaniasis using same preparation of paromomycin ointment. In this study, we undertook an evaluation of topical treatment for cutaneous leishmaniasis employing two concentrations, 10% and 2%, of paromomycin ointment without methylbenzethonium chloride. A total of 74 cutaneous leishmaniasis patients living in the village of San Sebastian (Ciento Tres), Department of Manabi, Ecuador, who agreed with informed consent were recruited for the present study. All the subjects continued their daily activity during the treatment without hospitalization. Follow-up of the treatment was performed between January and April, 1991. The treatment with 2% paromomycin ointment produced a marked improvement in five patients, a good reaction in 22, a slight reaction in 18 and no reaction in 10. When 2% paromomycin ointment was applied to the skin, ulcers started to dry but the induration tended to persist. The treatment with 10% paromomycin ointment produced a marked improvement in 4, a definite improvement in 10, slight reaction in four and no reaction in four. Some patients with large ulcerative lesions complained of a burning sensation during the application of 10% paromomycin, while those with plaque or nodules type of lesion did not. The present topial applications to the lesions of cutaneous leishmaniasis produced an relatively good results. In particular, almost complete healing of the lesions was obtained in several patients by topial treatment with paromomycin ointment alone. There have already been few reports on the topical use of paromomycin ointment. Paromomycin is usually used topically in high concentration such as 12.5%. Furthermore, it is used as an ointment in combination with methylbenzethonium chloride. We compared the effects between high (10%) and low (2%) concentration of paromomycin. In our preliminary study, some patients with large ulcerative lesions rejected to use our ointment because of burning sensation at the application of high concentration of the ointment. In a few case, low concentration also caused a slight burning sensation as well as high one. However, the degree was so mild that the patients could continue to use the ointment. We, therefore, selected a low concentration (2%) for large ulcerative lesions and a

high concentration (10%) for dry and nodulous and/or plaque-type of lesions.

61 ACCELERATED TRANSFORMATION OF *TRYRANOSOMA CRUZI* FROM TRYPOMASTIGOTE TO AMASTIGOTE IN A LOW pH MEDIUM

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The rate of transformation of *Trypanosoma cruzi* from trypomastigote to amastigote was accelerated in low pH medium. To determine whether this enhanced transformation follows its natural course, two events were investigated, namely: the ultrastructural changes in the kinetoplast and protein analysis. For electron microscopic studies, cells were collected at certain periods during transformation and processed using conventional methods. As has been documented by Inoki *et al.* (1971), De Souza (1984), and others, *T. cruzi* exhibits various forms of the kinetoplast at every stage in its life cycle. In agreement with them, we observed that the trypomastigote has a loose spacious kinetoplast wherein the component DNA fibrils are arranged irregularly. This kinetoplast form advances in regular compaction in the intermediate forms of the parasite and, eventually, develops into a rod-like highly coiled kinetoplast typical of the amastigote. However, a persistent observation was made on the existence of a kinetoplast form wherein the DNA fibrils are arranged in parallel rows. The number of rows, which is greatest in trypomastigotes, seemed to decrease in the course of transformation to assume the rod shape of the amastigote kinetoplast. Furthermore, in the event of this transformation process, protein was analyzed by SDS-PAGE and Western blot. The mouse anti-trypomastigote antiserum reacted with a major protein with a calculated MW of 68 kD. This major protein was observed to be greatest in trypomastigotes, reduced in transient intermediate forms, and absent in amastigotes. This protein, however, was observed to be present in epimastigote forms, too. In accordance with Saborio and his colleagues (1989), this trypomastigote-specific protein appears to be the paraaxial or paraflagellar rod protein. This was confirmed by IFA and IEM which localized the protein along the flagellum and on the complex lattice structure of the paraflagellar rod, respectively. From these observations, the trypomastigote-specific protein that appeared to decrease during transformation to amastigote is the paraflagellar rod protein. These results likewise suggest, as other authors have, the immunogenic property of this protein.

62 GENE ENCODING CYSTEINE PROTEINASE FROM *TRYPANOSOMA RANGELI*

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DNA fragments are obtained by the polymerase chain reaction (PCR) on genomic DNA from *Trypanosoma rangeli* using the oligonucleotide primers encoding the active site amino acids of cysteine proteinase. As cysteine proteinases of trypanosomes have been reported to be responsible for the invasion of the parasite to host cells, it is of great interest to compare the genetic variation of the gene among pathogenic and non pathogenic trypanosomes. After two step amplification by PCR, two different fragments, 475 and 498 bps, were finally obtained. These fragments were subcloned into plasmid vector for the subsequent sequence analysis and blot hybridization. The fragment of 498 bp length (Tr-CP) contains eight conserved amino acids in N- and C-terminal section of the representative eukaryotic cysteine proteinases. Overall

homology after alignment of the Tr-CP amino acids to the cysteine proteinases from the other trypanosome species were 63% to *T. cruzi* and 73% to *T. brucei gambiense*, respectively. The chromosome size patterns and the location of the gene were analysis by pulse-field gradient gel electrophoresis. *T. rangeli* has 14 chromosomes ranging from 350 Kb to 1.6 Mb, which were fewer in numbers and smaller sizes compared with those from *T. cruzi*. The Tr-CP gene hybridized with an equal intensity to chromosomal 1 (350 Kb) and chromosome 2 (470 Kb). The Tr-CP cross-hybridized with *T. cruzi* chromosomes about 1 Mb, although the intensity was much different. Northern blot analysis revealed that Tr-CP gene was specifically expressed in *T. rangeli* as 1.7 Kb mRNA.

These results may suggest that (1) the non-pathogenic *Trypanosoma* spp. contain the conserved gene corresponding to the cysteine proteinase or closely related enzymes, and that (2) there is more than one copy of this gene each found on different chromosomes.

63 SEARCH FOR THE PROTEINS THAT HAVE HIGH AFFINITY TO HIV-I NEF

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Nef is one of the non-structural genes encoded in the genom of human immunodeficiency virus (HIV). The protein product is associated with the cellular and plasma membrane, however its functional properties concerning viral replication and pathogenesis has been failed to demonstrate in tissue culture studies, since the viral replication *in vitro* is variably affected by mutations in Nef, and dependent on the host cell line.

However, an experiment performed *in vivo* using the simian immunodeficiency virus (SIV) revealed that rhesus monkeys, infected with the viruses generated from molecular clone which carries complete Nef, developed simian AIDS, in contrast, none of monkeys infected with Nef deleted viruses did.

To understand the mechanism which affects the viral pathogenesis *in vivo*, an attempt was carrird out to identify the host proteins which binds to Nef. First, the Nef or deletion mutant derived from HIV-1 Bru strain was fused to *Schistosoma japonicum* glutathione S-transferase (GST) gene, and the fusion proteins were produced. Then the glutathione bound chimera proteins were used to search for cellular proteins that could interact specificially with Nef. Second, using the purified ^{32}P-labeled GST-Nef or deleted Nef fusion protein, we performed the Far Western blot analysis of human T or monocytoied cell lines. We identified several proteins which carried relatively high affinity to Nef. Further characterization of those proteins will be discussed.

64 A MOLECULAR ANALYSIS OF THE V3 GENE OF HIV-I ISOLATED IN UGANDA

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The V3 loop in gp120 is one of the main targets for neutralizing antibody, antibody dependent cellular cytotoxicity and cytotoxic T-lymphocytes in the early phase of the HIV infection. The third variable region of the *env* gene has much diversity and it changes the characteristics as an epitope with only a single amino acid residue substitute in the V3 loop. It reduces initial immunological responses against HIV infection. Therefore, it is important to investigate the diversity of the *env* gene to make an effective multipotential vaccine

inducing good and broad immunological responses against HIV. We are now proceeding a collaboration study with Makerere University about the therapy of opportunistic infection in AIDS patients. We collected several serum samples at the capital of Uganda in 1991 and directly extracted viral RNA of 6 strains from the patient's serum employing Polymerase Chain Reaction (PCR) amplification method. The patients were one male and 5 female, and the mean age was 29.7. The PCR product was cloned into plasmid vector and sequenced. All of the strains obtained from the patients have same amino acid sequence GPGRAFV at the apex of the V3 loop. They have some mismatches around the apex region, but they almost resemble to the American European type and are different from the old African type already reported. We assume these strains may be imported from the neiboring countries or a result of immunological selection. All patients were suffering from pulmonary tuberculosis, so it may also affect the selection. In order to know the reason of type varitaion, we have to investigate more patients in several parts of Uganda.

65 A MECHANISM OF INHIBITION OF NATURAL KILLER CELL ACTIVITY LEADING TO AIDS OPPORTUNISTIC INFECTION

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Natural killer (NK) cell activity and lymphokine activated killer (LAK) cell activity (activated *in vitro* with IL-2) of peripheral blood lymphocytes (PBLs) were assayed from normal volunteers, AIDS asymptomatic carriers (A.C.) or AIDS patients. Among them, only NK cell cytotoxic activity was inhibited exclusively in AIDS patients. PBLs from A.C. showed normal NK activity and LAK activity was intact during the entire course of this disease. The results showed that the patients are still in A.C. state (no onset of opportunistic infection yet) even if the $CD4^+$ lymphocytes are zero in PBLs as long as the NK cell activity was intact. And they suggested that the NK cells as well as $CD4^+$ T lymphocytes seem to be responsible for the susceptibility of opportunistic infections in AIDS patients. To know the mechanism of suppression of NK cytotoxic activity, we first prepared synthetic 17 mer peptides conserved in retroviruses. CKS17 peptide equivalent to p15E of retroviral envelope protein effectively inhibited NK activity and LAK activity of PBLs from A.C. and normal volunteers. On the contrary, plasma from AIDS patients inhibited NK activity but not LAK activity. These results are fit to be the cellular immunity of AIDS patients. This plasma did not contain HIV RNA detected by reversed PCR assay. In summarized, we want to stress the significance of natural immunity to protect the onset of opportunistic infection in AIDS patients and some mechanism of impairing NK cell cytotoxic activity in AIDS plasma seems to be responsible for this.

66 TREATMENT OF OROPHARYNGEAL CANDIDIASIS AND CRYPTOCOCCAL MENINGITIDIS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)-SEROPOSITIVE PATIENTS IN UGANDA

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We are now developing a collaborative project titled "Treatment of AIDS-related infections in Uganda". In this paper, we demonstrated ongoing data on the studies of treatment with amphotericin B syrup for human immunodeficiency virus (HIV)-seropositive patients with oropharyngeal candidiasis and treatment with oral fluconazole for these patients with cryptococcal menigitis.

Thirty one patients (mean age: 30.7 y.o.) with oropharyngeal candidiasis were invited to the trial. 96.8%, 54.8%, and 64.5% of patients showed weight loss (>10% of BW), fever (>1 month) and diarrhea (>1 month). Nine *Candida albicans* strains were isolated from patients. Clinical responses to the drug were evaluated according to the oral findings and clinical symptoms (swallowing pain). The treatment with amphotericin B syrup (1,200 mg per day) for two weeks provided an efficacy rate of 67.7%. No reverse effects were noted. These data suggest the clinical usefulness of amphotericin B syrup for oropharyngeal candidiasis in HIV-seropositive patients.

Ten patients (mean age: 31.9 y.o.) with cryptococcal meningitis entered the trial. The diagnosis was given by testing cryptococcal staining with India ink and cryptococcal antigen in cerebrospinal fluid (CSF) from patients. Cryptococcal culture was also done. Three *Cryptococcus neoformans* were isolated. All the patients showed weight loss (>10% of BW), fever (>1 month), and oral candidiasis. Four patients responded to the treatment with fluconazole (200 mg or 400 mg per day) for 8 weeks. In these cases, the maintenance therapy with low dose fluconazole (200 mg per week) is given for 4 months. No reversed effects were observed. However, 6 patients did not responded to the therapy and died due to meningitis within a week after the initiation of the therapy. The delay of diagnosis and therapy for this disease appears to lead HIV-seropositive patients to death.

In summary, these preliminary data suggest the clinical usefulness of amphotericin B syrup for oropharyngeal candidiasis and oral fluconazole for cryptococcal meningitis in HIV-seropositive patients. These studies requires further investigations.

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