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DISEASES AND LABORATORY FINDINGS OF JAPANESE STAYING LONG PERIODS IN TROPICAL AND SUBTROPICAL COUNTRIES —THREE YEAR STUDY ON THE JAPAN OVERSEAS COOPERATION VOLUNTEERS

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Abstract: With the purpose of investigating the major diseases and the main problems in health management among the Japanese staying for a long period in tropical and subtropical countries, an epidemiological survey was made on the diseases and clinical laboratory data of the Japan Overseas Cooperation Volunteers (JOCV) who lived in and extended technical cooperation to the developing countries. Of the major health problems found in a total of 1,469 subjects (1,085 males and 384 females), malaria (101 cases), acute hepatitis (94 cases), chronic diarrhea/enteritis (78 cases) and traffic accidents (71 cases) ranked high. Suspecting that many cases of malaria and dengue fever were not reported, further investigation was carried out and it was revealed that the incidence of clinical malaria ranged from 38.1 to 66.7 per cent in tropical African countries. The incidence of malaria was 36.5 per cent in those who regularly took a combination of sulfamonomethoxine-pyrimethamine while it was 50.4 per cent in those who took the combination irregularly or not at all. Many cases with intestinal parasites (30.7%), eosinophilia (5.9%), and abnormal hepatic function (5.4%) were detected upon their return to Japan. The incidence of mental disorders and venereal diseases was suspected to be considerably higher than the number of cases reported. Among the so-called "tropical diseases", a high incidence of malaria and infectious diseases of the digestive organs was observed. Prevention of those diseases is, needless to say, important but in view of the high rates of hepatitis, mental diseases, venereal diseases, traffic accidents and aggravation or recurrence of chronic diseases which were present before their overseas assignment, it is also important to take steps to prevent such problems when Japanese, especially young people, stay in tropical or subtropical countries.

INTRODUCTION

Recently the number of Japanese staying for long periods in tropical or subtropical countries is constantly increasing and the importance of knowledge of tropical diseases and health management for those Japanese staying in such countries has been pointed out (Owri, 1982; Ohara *et al.*, 1982).

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In tropical and subtropical countries there are diseases which is not in Japan but peculiar to those countries, such as malaria, yellow fever, and cholera or those diseases of bilharziasis, onchocerciasis, and Chagas' disease. These diseases constitute a serious menace to Japanese who stay in those countries. Prevention of these diseases is extremely important when Japanese stay in tropical or subtropical countries. But the diseases which cause problems to Japanese staying in those countries are not limited to the above mentioned tropical diseases. Mental diseases, traffic accidents, although these are not necessarily so-called tropical diseases, and the system under which health management is carried out seems to be also important.

Diseases and laboratory findings of young Japanese staying in tropical and subtropical countries were studied in the present paper. By collecting data on the diseases the subjects had suffered during their overseas stay, on the results of the health examination they underwent upon their return to Japan, and on health conditions during their stay in those countries, the authors want to make observations on the diseases Japanese are apt to suffer and the problems which are important concerning their health management during a long stay in those countries.

Materials and Methods

The subjects were members of the Japan Overseas Cooperation Volunteers (JOCV). They were volunteers aged between 21 and 35, who had been dispatched to developing countries (27 countries at March of 1982, Figure 1) with the purpose of contributing to economic and social development. Staying in tropical or subtropical countries, they offered a variety of technical cooperation. They lived in many

Figure 1 Countries where the members of the Japan Overseas Cooperation Volunteers are dispatched (March, 1982).

places of each country where the sanitary conditions were extremely poor.

The period for investigation was the three years from April 1979 to March 1982. Based upon the "Report Concerning the Occurrence of Injuries and Diseases" sent to JOCV headquarters in Tokyo from the offices in all the countries concerned, and the results of the health examinations conducted before their departure from Japan and on their return home, statistical analysis was carried out. There were 1,469 subjects (1,085 males and 384 females) who lived in those countries for more than three months during the three years under investigation, and 815 (604 males and 211 females) who returned to Japan during those three years. The number of JOCV members who stayed in those countries in the middle of 1979, 1980 and 1981 were 706, 840 and 931 respectively.

Concerning malaria and dengue fever, an investigation was carried out separately by means of a questionnaire given to 282 subjects (209 males and 73 females) and 122 (94 males and 28 females) respectively among those who returned to Japan from the year of 1980 to 1981. In order to grasp the condition of affliction more precisely, the reports available from each country was also used.

Serological testing for total hepatitis A virus antibodies (anti-HAV) was performed using commercial radioimmunoassay kits (HAVAB, Abbott Laboratories, North Chicago, U. S. A.). Anti-HAV was determined positive when the inhibition titer was more than 70 per cent. Serological testing for hepatitis B surface antigen (HBsAg) and antibody for HBsAg (anti-HBs) both before and after assignment was also performed as one of the routine health examinations.

To investigate the health complaints and venereal diseases incurred during their stay in those countries, we analyzed statistically the data available to us through treating and questioning 96 volunteers (81 males and 15 females) in the course of our on-the-spot examinations (made from December 1981 to January 1982 in Bangladesh, Ghana, Liberia, Morocco and Tunisia).

Concerning the reports we received from those countries, endeavours were made to cover all the cases without omission, and all the cases requiring medical treatment were included in the reports, but it is supposed that there were many unreported cases of mild diseases not requiring medical treatment (such as cold, mild diarrhea, or low grade pyrexia).

Results

Major diseases: The number of cases of major diseases (excluding those of parasitosis, respiratory infections and dental diseases) which occurred in those three years and the distribution of major diseases among the regions are shown in Table 1 and 2 respectively. Those ranking high are malaria with 101 cases, acute hepatitis 94, chronic diarrhea/enteritis 78, traffic accidents 71, gastritis/peptic ulcer 57, dermal diseases 50, trauma excluding traffic accidents 36, followed by lumbago, urinary tract infection/nephritis, ophthalmic diseases, nasal diseases and food poisoning. Other internal diseases include anemia, tuberculous pleulitis, cholelithiasis, cases suggesting nocardiasis and others. In cases of bone fracture and trauma, about 60 per cent were caused by traffic accidents, but there were also cases of fracture caused by falling

Table 1 Major diseases among the members of the Japan Overseas Cooperation Volunteers (1979–1981)

Diseases	'79	'80	'81	Total	Rate per year per 1,000
Malaria	17	20	64	101	40.8
Acute hepatitis	31	36	27	94	37.9
Chronic diarrhea/enteritis	11	22	45	78	31.5
Traffic accidents	28	19	24	71	28.7
Gastritis/peptic ulcer	14	13	30	57	23.0
Dermal diseases	15	19	16	50	20.2
Trauma	5	15	16	36	14.5
Lumbago	6	9	10	25	10.1
Urinary tract infection/ nephritis	4	8	7	19	7.7
Ophthalmic diseases	4	9	5	18	7.3
Nasal diseases	4	10	4	18	7.3
Food poisonings	8	4	4	16	6.5
Venereal diseases/urethritis	5	2	6	13	5.2
Mental diseases	4	4	4	12	4.8
Appendicitis	5	4	3	12	4.8
Dengue fever	3	4	5	12	4.8
Typhoid fever	2	2	2	6	2.4
Other internal diseases	10	24	22	56	22.6
Total	176	224	294	694	279.4

The number of members in mid 1979, 1980 and 1981 was 706, 840 and 931 respectively.

Table 2 Distribution of major diseases in each region (%)

Diseases	Southeast Asia	Middle East North Africa	East Africa	West Africa	South America
Hepatitis	46/444 (10.4)	9/115 (7.8)	18/561 (3.2)	9/110 (8.2)	12/239 (5.0)
Traffiic accidents	23/444 (5.2)	5/115 (4.3)	29/561 (5.2)	7/110 (6.4)	7/239 (2.9)
Intestinal infections	38/444 (8.6)	9/115 (7.8)	10/561 (1.8)	15/110 (13.6)	6/239 (2.5)
Malaria	3/26 (11.5)	—	67/119 (58.0)	9/20 (45.0)	—
Dengue fever	20/122 (16.4)	—	—	—	—
Parasite (+)*	133/297 (44.8)	29/96 (30.2)	47/267 (17.6)	9/17 (52.9)	12/73 (16.4)

Numerators indicate frequency of the disease and denomenators the number of people who stayed in each region for more than three months.

* Yamaura *et al.*, 1981.

from a horse and by pistol-shots in the lower thigh during an outing at night. The 12 cases given there as mental diseases were all highly advanced, but it is supposed

that mild mental affections were manifested in a great many members.

Of the 71 cases of traffic accidents, collision while riding a motorbike accounted for 65 per cent, and 77 per cent of them occurred at night. The cases which required treatment in Japan totaled 13.

A fairly high number complained of symptoms of diarrhea after their arrival in a tropical or subtropical country. As the cause of diarrhea, mental or physical fatigue resulting from changes in the environment of daily life and simple diarrhea due to a change in water quality were given. Besides these reasons, bacterial diarrhea, amoebic dysentery or giardiasis were frequently observed. There were many cases which turned chronic after frequent repetition of diarrhea following the onset of acute symptoms.

Malaria: Of the 282 subjects in our investigation on malaria, those who were regarded as having stayed in malaria endemic districts during their duty were 165. Statistical analysis was performed on these 165. The results revealed that those whose disease was diagnosed as malaria by physicians or whose disease was strongly suspected of being malaria from their clinical symptoms were found particularly in East and West Africa. The incidence of malaria among those who stayed for two years in the endemic districts was 66.7 per cent in Malawi, 62.5 per cent in Tanzania and 50.0 per cent in Kenya (Table 3). Among the subjects in the investigation, only three cases (in the Philippines) contracted the disease in places other than Africa. A small number of the patients were found in Central and South America, but they were excluded in the present study.

Table 3 Incidence of malaria among the Japan Overseas Cooperation Volunteers

Country	Total No. of volunteers	Malaria (+)	Incidence (%)
Malawi	48	32	66.7
Tanzania	16	10	62.5
Kenya	34	17	50.0
Ghana	20	9	45.0
Zambia	21	8	38.1
Philippines	26	3	11.5
Total	165*	76	46.1

The diagnosis was made clinically in the majority of the patients.

* Of the 282 patients examined, 165 people were regarded as having stayed in malaria endemic districts.

For prevention of malaria, a weekly dose of two tablets of a combination of sulfamonomethoxine 250 mg and pyrimethamine 12.5 mg (MP tablet) was recommended (chloroquine was not available when they left Japan). The results of our investigation on the effect of the above medication revealed that no outbreak was seen among those who took the combination regularly in countries other than African countries. But in East and West African countries, the number of cases of malaria in

spite of regular ingestion was noticeable. The incidence of malaria was 38.2 per cent (13/34) in East Africa, 50.0 per cent (6/12) in West Africa, and 41.3 per cent (19/46) in Africa as a whole among those who took the agent regularly. Observations made on subjects divided into those of regular ingestion and of irregular ingestion for comparative study of efficacy, showed that incidence was lower in the group of regular ingesters than among irregular ingesters and non-ingesters, but the difference was not significant (Table 4).

Table 4 Preventive effect of sulfamonomethoxine-pyrimethamine mixture (regular ingestion vs. irregular or none)

Area	Africa			Africa and Philippines		
Ingestion	Regular	Irregular or none	Total	Regular	Irregular or none	Total
Malaria (+)	19 (41.3)	54 (58.1)	73 (52.5)	19 (36.5)	57 (50.4)	76 (41.3)
(−)	27 (58.7)	39 (41.9)	66 (47.5)	33 (63.5)	56 (49.6)	89 (53.9)
Total	46	93	139	52	113	165
	$X^2=3.47$ $0.10>P>0.05$			$X^2=2.77$ $0.20>P>0.10$		

( ): %

Hepatitis: 94 cases of acute hepatitis were reported in the past three years in all the countries and the incidence was 37.9 per year per thousand persons.

Serological studies carried out on 94 cases of acute hepatitis have revealed that 73 cases (77.7%) were strongly suspected to be hepatitis A, however there were nine cases of hepatitis B, and two cases of non A non B hepatitis have been confirmed (Table 5). In November 1980 there was a mass outbreak of hepatitis A in the Philippines caused by ingestion of raw oysters (Ohara *et al.*, 1983). Hepatitis usually developed from six months to one year after arrival in the country.

Table 5 Types of 94 cases of hepatitis

Types	Cases	%
A	73	77.7
B	9	9.6
Non A Non B	2	2.1
Unknown	10	10.6
Total	94	100.0

Dengue fever: 12 cases of dengue fever have been reported during the past three years but the actual incidence of the disease among the members stationed in South East Asia and Oceanic countries was estimated to be much higher although the majority of the cases might be mild ones. In the present study, those who were diagnosed as having dengue fever by local physicians and whose symptoms strongly suggested

Table 6 Incidence of dengue fever among the Japan Overseas Cooperation Volunteers

Country	Total No. of volunteers	Dengue fever (+)	Incidence (%)
W. Samoa	15	4	26.7
Philippines	46	12	26.1
Tonga	4	1	25.0
Malaysia	35	2	5.7
Bangladesh	22	1	4.5
Total	122	20	16.4

The diagnosis was made clinically in the majority of the patients.

of dengue fever were 20 persons among 122 examinees (16.4%) (Table 6).

Complaints: Many people have various kinds of complaints during a long stay in tropical and subtropical countries. The investigation was carried out on 96 members of the JOCV who were staying in those countries (the average period of stay was 346 days) by means of personal interview. Results showed that those who complained of easy fatigability accounted for 62.5 per cent, followed by decrease of body weight (34.4%), mental fatigue (22.9%) and loss of appetite (14.6%). The worse the climate and sanitary conditions were, the higher was the rate of those who had such complaints (Table 7).

Table 7 Complaints of the members during their stay in tropical countries

Complaints	No.	%
Easy fatigability	60	62.5
Decrease of body weight	33	34.4
Mental fatigue	22	22.9
Loss of appetite	14	14.6
Amenorrhea	2	13.3
Diarrhea	5	5.2
Decrease of visual acuity	3	3.1
Stomachache	2	2.1
Toothache	2	2.1
Alopecia	2	2.1

96 volunteers were examined (81 males and 15 females).

Laboratory findings: The results of the health examination carried out by the Institute of Medical Science at the University of Tokyo upon their return to Japan are shown in Table 8 (the stool examination was undertaken by the Department of Parasitology of Tokyo Women's Medical College).

Among abnormal findings, parasites were most frequent, seen in 30.7 per cent of all the examinees. When broken down, *Giardia lamblia* was the most common of pro-

Table 8 Abnormal laboratory findings in the returned volunteers

Finding	No.	%
Parasite (+)**	230	30.7
Eosinophilia*	48	5.9
Abnormal hepatic function*	44	5.4
Urethritis*	42	5.2
HBs antigen (+)*	34	4.2
HBs antibody (+)	101	12.4
Elevated ESR*	29	3.6
Anemia*	16	2.0
Urine protein (+)*	13	1.6
Serological test for syphilis (+)*	9	1.1
Urine sugar (+)*	9	1.1
Abnormal thoracic shadow*	5	0.6

*: 815 volunteers were examined.
**: 750 volunteers were examined.

tozoal diseases, seen in 17.3 per cent of all the examinees, followed by *Entamoeba coli* 2.1 per cent and *Entamoeba histolytica* 1.6 per cent. Among helminthic diseases *Trichuris trichiura* formed 9.3 per cent and *Ascaris lumbricoides* 3.7 per cent (Yamaura *et al.*, 1981). In 1980 a case of bilharziasis was reported in Zambia. Of cases of eosinophilia, those attributable to helminth infection acounted for 88 per cent.

Among 44 cases in which abnormal hepatic function was detected, cases in which apparent symptoms of acute hepatitis were not present and abnormalities were detected for the first time by hepatic function test were seen, in addition to cases in which hepatic function had not normalized after the occurrence of acute hepatitis. The cause is not known in detail, but it is supposedly attributable to drug induced liver injury, dysfunctions caused by parasitosis such as *Giardia lamblia*, or influence of food or drinking water.

Of 815 examinees, there were 34 persons with HBsAg (+) and 101 with anti-HBs (+). Out of these 815 persons 375 could be compared using paired serum and the results are shown in Table 9. Those whose HBsAg and anti-HBs turned from (−) to (+) during their stay in each country were three and 13 respectively. The incidence of people whose anti-HAV was positive also increased after staying in those countries although the antibody titer could not be measured with paired serum.

Complaints of lumbago, dermal diseases and venereal diseases-like symptoms were found at fairly high rates. As dermal diseases, micotic dermatitis and bites by bloodsucking insects were detected rather frequently.

Comparatively few cases of venereal disease were actually reported but a fairly large number had in fact contracted it or were suspected of contracting it. The survey made by means of questionnaires gives to 100 males disclosed that those who had subjective symptoms suggesting venereal diseases amounted to 28 per cent and those who had ingested antibiotics due to suspicion of venereal diseases 18 per cent. The cause of venereal diseases-like symptoms such as micturition pain, excretion of

Table 9 Serological study on hepatitis virus infection of the volunteers

	Positive case (%)		Case of conversion	
	before	after	+→−	−→+
HBsAg	17 (4.5)*	19 (5.1)*	1	3
HBsAb	32 (8.5)*	44 (11.3)*	1	13
HAAb	41 (11.0)**	79 (29.4)***	?	?

*: 375 persons were examined.
**: 374 persons were examined.
***: 269 persons were examined.

pus and enlargement of the inguinal lymph nodes, was mostly non-gonococcal urethritis, and those presenting such symptoms are increasing. But there were few afflicted with the classical venereal diseases such as syphilis, gonorrhea or chancroid.

Past history and present illness: Following up those who had contracted diseases before leaving Japan showed that in their new environment they had a high incidence of diseases obviously related to them. By way of illustration, among the major diseases which developed and required treatment during their stay in the developing countries, diseases contracted in the past were found in 38.5 per cent of the cases of gastritis/peptic ulcer, 31.3 per cent of lumbago, 50.0 per cent of bronchial asthma, 97.6 per cent of dental diseases and 44.4 per cent of nasal diseases; there were many who had no alternative but to return to Japan, as treatment for their diseases was unavailable in those countries (Table 10).

Table 10 Relationship between the past history and the present illness; retrospective study

Present illness	Cases	Past history (+)	
		Recurrence (+) (%)	Recurrence (−)
Dental caries	84	82 (97.6)	143
Gastritis, peptic ulcer	13	5 (38.5)	16
Lumbago	16	5 (31.3)	12
Sinusitis	9	4 (44.4)	12
Pyelonephritis	4	2 (50.0)	2
Hypertention	3	3 (100.0)	2
Cholelithiasis	2	1 (50.0)	3
Bronchial asthma	2	2 (50.0)	0

1,469 persons were examined.

Discussion

Collection and statistical analysis of the data were performed as to the main diseases which had occurred in JOCV members who stayed long in tropical and

subtropical countries in the past three years. If we look at major infectious diseases, a remarkably large number of the members contracted orally-infected diseases such as infectious diseases of the digestive organs caused by various kinds of pathogens, malaria and other mosquitoborne diseases; and dermatomycosis. This suggests the extreme importance of measures to prevent such diseases among members when they stay in tropical and subtropical countries.

It is easy to imagine that the poor sanitary environment causes oral infections in many, but above all, hepatitis A is the most serious disease. Recently in Europe and America (Kendrik, 1972; Kendrik, 1974; Skidmore *et al.*, 1982) as well as in Japan (Imai *et al.*, 1978; Obata *et al.*, 1978), hepatitis A has been regarded as a disease very common among young people who stay in tropical and subtropical countries. Among the JOCV's young members, its incidence tends to rise year by year. Of late the sanitary environment in Japan has improved and at the same time a sharp drop is seen in the number of carriers of antibodies for hepatitis A virus (anti-HAV) among young people (Ministry of Public Health, Japan, 1979). It is, therefore easily imagined that a generation which has grown up in an environment in which hepatitis type A virus (HAV) is not prevalent will very easily be afflicted with hepatitis A when they stay in countries where such virus is prevalent. Most natives, on the other hand, become carriers of antibody in their childhood (Sato, 1981; Wankya and Hansen, 1979), and it is supposed that they present no symptom or only cold-like symptoms when they actually contract hepatitis A, which is not regarded as a very serious disease among them. As a readily available vaccine has not yet been produced, it is necessary to take preventive measures by means of gamma-globulin injections (Trond, 1963).

It is reported that there are many chances of contracting hepatitis B during stays in tropical and subtropical countries (Marklein *et al.*, 1980), and in the results of the present investigation, an increase was seen in the rates of harbouring both HBs antigens and antibodies after staying for two years. As the route of infection, filthy medical instruments, sexual intercourse and others are assumed.

Among orally-infected diseases other than hepatitis A, infectious diseases in the digestive organs caused by various pathogens are seen in many cases, but they are generally mild diseases, mostly being only transient diarrhea or abdominal pain. It is imagined that there are a considerable number of members who experienced so-called traveller's diarrhea. Typhoid fever has been reported in six cases in the past three years, but all the patients were cured entirely by the treatment given them in the local hospitals. On their return to Japan, 30.7 per cent were found to have parasite ovas in the stool examination. But helminthicidal procedures were taken in some countries for those in whom parasitization was detected during the health examinations carried out twice a year, so the actual rate of parasitic infection must have been higher.

Malaria was detected in countries in West and East Africa, Southeast Asia, and Central and South America. In Africa, above all, it was clear that an extremely high rate of contraction was registered in the present investigation as well as in other reports (WHO, 1979); as most of the cases are falciparum or malignant malaria, it is a serious problem for those who stay in those African countries where malaria is

prevalent. Investigation into the preventive effect of ingesting a combination of sulfamonomethoxine and pyrimethamine suggested that the effect could not be regarded as having been satisfactorily confirmed. It is necessary to take proper steps to counter the malaria found in each country, taking into consideration drug tolerence (Ebisawa *et al.*, 1979), side effects, the prevalence of malaria in each country, the species of malaria and other factors.

Concerning dengue fever, few cases were reported on account of the majority being mild, but it is supposed from the present investigation that there are a considerable number who had actually been afflicted with this disease in Southeast Asian and Oceanic countries.

Among diseases which many natives are confirmed as suffering from, malaria and relatively mild infectious diseases of the digestive organs are also the disorders which hit many Japanese during their stay there. But the volunteers scarcely suffered from the diseases against which vaccination is enforced (preventive injections are given to JOCV members against yellow fever, cholera, tetanus, rabies, poliomyelitis and Japanese encephalitis). Almost no cases were detected of tropical diseases prevalent only in small limited areas i.e. diseases of endemic nature. It is, therefore, suggested that the contraction of tropical disease is prevented by proper hygienic measures exercised in each country where the disease is prevalent.

On the other hand, as seen in hepatitis A, it is striking to see how frequently Japanese catch diseases for which they have no antibodies when they enter countries where pathogenic organisms are widely prevalent. The high incidence of traffic accidents, mental diseases, venereal diseases, and recurrence or aggravation of diseases contracted previously is also noteworthy. These may be termed problems which scarcely give rise to public awareness among the natives. They cannot necessarily be called tropical diseases, but as a matter of fact, they may be said to be very serious for the Japanese staying in tropical or subtropical countries. Proper ways of countering them seem to have been neglected while steps to prevent so-called tropical diseases have been taken.

To an unimaginable extent bitter cultural friction is experienced by those who enter countries in which the living environment, climate and customs are all different from those in Japan (Inamura, 1980), and the greater part of them are said to have been seized with mild neurosis-like symptoms when they arrived in those countries. Many of them become adapted to such environments as time goes on, but there are often persons in whom such adaptation cannot be made and various kinds of noticeable mental reactions appear, or schizophrenia becomes manifest. A sharp increase is seen in traffic accidents on account of unfamiliarity with traffic conditions and lack of geographical knowledge of each place; in addition there are many cases of drunken driving from a feeling of freedom or shortage of amusement facilities.

There are many cases of aggravation or recurrence of diseases acquired in the past on account of the influences exerted by various kinds of factors such as harsh climate, work stress, difficulties in treating chronic diseases in a poor medical care system and others. Also when they are afflicted with diseases in those countries, many are forced to return to Japan due to difficulties in receiving satisfactory treatment resulting from many adverse therapeutic conditions such as poor medical care system,

dietary differences and linguistic problems. When we think of those problems, we are made to feel strongly the necessity of examination before their departure from Japan, or of their departure only after receiving complete treatment if any abnormalities are detected in such examinations.

Summary

The diseases and accidents which the Japan Overseas Cooperation Volunteers suffered from during their two years of assignment overseas in tropical and subtropical countries were analyzed and the following conclusions were obtained.
1) Endemic diseases such as malaria, hepatitis, diarrheal diseases, dengue and other arbo-virus diseases are important and frequent diseases among the Japanese staying in tropical or subtropical countries. Efforts should be made to give the youths instructions in preventing them before their departure to those countries.
2) Knowledge about and ways of preventing traffic accidents, mental health and the recurrence of other non-tropical diseases which the youths had suffered in the past, such as peptic ulcer, dental caries, hypertension, and neurotic diseases should be given equal emphasis.
3) The conclusions and suggestions apply not only to the JOCV but also to other Japanese or subjects in non-endemic, well developed countries who go to tropical and subtropical countries and stay there for some time.

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熱帯亜熱帯地方長期滞在日本人の疾病と検査成績

—過去3年間における青年海外協力隊員を対象とした健康調査

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熱帯亜熱帯地方長期滞在日本人の疾患罹患状況を把握し，健康管理上の重要事項について考察するために，開発途上国に3カ月以上滞在し技術協力を行う青年海外協力隊員を対象に，滞在中に発生した主な疾患と検査成績につき集計し分析した。調査の対象となったのは総数1,469名（男1,085名，女384名），調査期間は，1979年4月から1982年3月までの3年間である。

報告された主要疾患の内訳では，マラリア101例，急性肝炎94例，慢性下痢症・腸炎78例，交通事故71例が上位を占めていた。マラリア，デング熱については未報告例も多いと考えられ，さらに詳細な調査を実施したところ，流行地では，それぞれ38.1–66.7%，4.5–26.7%の罹患率であることが判明した。sulfamonomethoxine と pyrimethamine の合剤を規則的に予防内服していた者のマラリア罹患率36.5%に対し，不規則的及び非服用の者では50.4%であったが，両者に有意差は認められなかった。

性病，精神疾患は，それぞれ13例，12例が報告されたが，実際の罹患率はさらに高率であることが予想された。帰国時検査の異常所見として，糞便検査の結果寄生虫陽性例30.7%（山浦ら，1981），好酸球増多症5.9%，肝機能異常5.4%などが高率であった。また，現地滞在中に発生した疾患の中には，出国前に既往歴を有する例の悪化または再発と考えられるものも多く認められた。

日本人が熱帯亜熱帯地方に長期間滞在する際には，いわゆる“熱帯病”といえる疾患の中でも，マラリア，デング熱，寄生虫などによる消化器感染症等の罹患率が高いことが明らかにされたが，急性肝炎，交通事故，精神病，既往歴のある疾患の悪化または再発例も多く，熱帯病同様にこれらの疾患に関する対策も極めて重要であることが示唆された。

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SUSCEPTIBILITY OF *SIMULIUM METALLICUM* TO INFECTION WITH *ONCHOCERCA VOLVULUS* IN VENEZUELA[1]

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Abstract: The degree of susceptibility of *Simulium metallicum* to infection with *Onchocerca volvulus* was studied in Venezuela. Wild flies which had fed on the infected volunteer were maintained at a temperature varying between 22°C and 28°C, and all flies dying every 24 hours were dissected for larval development. Third-stage larvae were first seen in the head of flies dying between five and six days. Few abnormal and deformed larvae were observed. The larval development was asynchronous; only 41 of 117 larvae recovered from 32 flies examined on days 6–11 were in the third stage, the remaining larvae being still in earlier stages. However, the proportion of third-stage larvae among all larvae recovered increased from 6 to 69 per cent, with the passage of time from days 6–8 to 9–11. Likewise, the percentage of third-stage larvae found in the head during the same period rose from 25 to 54 per cent. These results indicate that despite the asynchronous and retarded development, many larvae can develop to the infective stage in *S. metallicum* and the possibility of infective larvae capable of being eventually inoculated into man is high, when flies could survive more. It is suggested that the susceptibility of *S. metallicum* in Venezuela to the indigenous strain of *O. volvulus* is relatively high, as compared with that of the same species in Guatemala or Colombia.

INTRODUCTION

Peñalver (1961) regarded for the first time *Simulium metallicum* Bellardi as the principal vector of onchocerciasis in the northern foci of Venezuela. Lewis and Ibáñez de Aldecoa (1962) assessed the vectorial capacity of this species, as well as *S. exiguum* Roubaud, from their field observations. They concluded that *S. metallicum* was the only important vector in these regions. Duke (1970) made the experimental infection and compared the developmental potential of *Onchocerca volvulus* microfilariae

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ingested by these two blackfly species. Ramírez Pérez *et al.* (1976) described the morphological features of *O. volvulus* larvae developing in *S. metallicum*. However, no data were available on the degree of susceptibility of this blackfly species to infection with *O. volvulus* in Venezuela. Apparently such information is needed to compare the vectorial capacity of *S. metallicum* in Venezuela with that of the same species in Guatemala or Colombia, where this species has been reported to be less important as a vector (De León and Duke, 1966; Collins, 1979; Ito *et al.*, 1980; Tidwell *et al.*, 1980).

In the present experiment, the degree of susceptibility of the Venezuelan *S. metallicum* to the indigenous strain of *O. volvulus* was assessed, mainly by means of the proportion of third-stage larvae among all larvae recovered from the flies which survived beyond the time when larvae became infective, as well as that of surviving flies positive for third-stage larvae.

Materials and Methods

The experimental infection was carried out in November 1982, at Guanaguana in Monagas, one of the onchocerciasis-endemic foci in the north-eastern region of Venezuela. The general information on this village or its surrounding areas was previously reported by Lewis and Ibáñez de Aldecoa (1962). The predominant man-biting blackfly species during the present study was *S. metallicum*, comprising 95 per cent of the total catches.

Wild females of *S. metallicum* were allowed to feed on the lower half of the legs of a human volunteer with low microfilarial density — two microfilariae per skin snip from a calf, taken with corneoscleral punch (Holth type). Simultaneously, engorged blackfly females were captured from a control volunteer without microfilariae. All these blood-fed flies were collected and maintained individually in a polypropylene tube, using a method previously described (Takaoka *et al.*, 1982). All flies were kept at a temperature varying between 22°C and 28°C, dead flies being removed everyday thereafter and stored in 70 per cent ethanol solution for later dissection. The number of dead flies was recorded to compare daily probabilities of survival in the groups of flies which had fed on the infected and control persons, respectively. All preserved specimens were divided into head, thorax and abdomen and dissected in a drop of five per cent Giemsa solution on a glass slide under a binocular stereoscopic microscope. The number of larvae in each part of the body was counted and their stages of development were determined by the size and morphological features, as defined by Duke (1968). To assess microfilarial intake, 20 flies which fed on the infected volunteer were killed immediately after feeding, and dissected using a technique by Nakamura (1964).

Results

Since wild flies were used in this study, it is possible that natural infections were mixed in the results. According to Lewis and Ibáñez de Aldecoa (1962), 3.9 per cent of wild-caught *S. metallicum* were naturally infected with *O. volvulus* larvae in the

Table 1 Larval development of *Onchocerca volvulus* in *Simulium metallicum* at 22–28°C

Days post-infection	No. flies with any stage of larvae/No. flies dissected (%)	No. larvae per positive fly	No. flies with 3rd-stage larvae/No. flies with any stage of larvae (%)	No. 3rd-stage larvae per positive fly	No. flies with 3rd-stage larvae in the head/No. flies with 3rd-stage larvae (%)	No. 3rd-stage larvae in the head per positive fly
		Mean (Range)		Mean (Range)		Mean (Range)
0	8/20 (40)*1	4.5 (1–10)	—	—	—	—
1–5	13/25 (52)	9.8 (1–59)	—	—	—	—
6–8	20/72 (28)	3.2 (1–19)	4/20 (20)	1 (1)	1/4 (25)	1 (1)
9–11	12/39 (31)	4.2 (1–12)	10/12 (83)	3.6 (1–11)	6/10 (60)	3.3 (1–7)
Total*2	32/111 (29)	3.4 (1–19)	14/32 (44)	2.9 (1–11)	7/14 (50)	3.3 (1–7)

*1 These flies were killed immediately after feeding to assess microfilarial intake.

*2 Total from days 6–11.

Table 2 Number and proportion of each developmental stage of *Onchocerca volvulus* larvae in *Simulium metallicum* which died on days 6–11 after ingestion of an infected blood-meal

Days post-infection	No. (%) larvae of				
	Any stage	Microfilarial stage	First stage	Second stage	Third stage
6–8	63	0 (0)	31 (49)	28 (45)	4 (6)
9–11	54	1 (2)	11 (20)	5 (9)	37 (69)
Total	117	1 (1)	42 (36)	33 (28)	41 (35)

Guanaguana area. Therefore, the rate of experimental infection shown in Table 1 was assumed to be slightly overestimated.

Under a temperature varying between 22°C and 28°C, third-stage larvae were first seen in the head of female *S. metallicum* dying between 5 and 6 days after ingestion of microfilariae. Few abnormal and deformed larvae were observed. The development of *O. volvulus* larvae in *S. metallicum* was asynchronous, as evidenced by the fact that third-stage larvae were found in 44 per cent (14/32) of the flies harboring any stages of larvae examined on days 6–11 (Table 1). In other words, only 41 of 117 larvae recovered during the same period were in the third stage, while the other larvae remained in earlier stages (Table 2).

However, as shown in Table 1, the proportion of flies with third-stage larvae among all flies positive for any stage of larvae increased from 20 to 83 per cent, with the passage of time elapsing from days 6–8 to 9–11. The proportion of third-stage larvae among all larvae recovered during the same period also increased from 6 to 69 per cent (Table 2).

Likewise, the proportion of flies with third-stage larvae in the head augmented from 25 to 60 per cent (Table 1); and the percentage of third-stage larvae found in the head rose from 25 to 54 per cent, as the time passed from days 6–8 to 9–11 (Table 3).

Table 3 Distribution of third-stage larvae of *Onchocerca volvulus* in the body of *Simulium metallicum* which died on days 6–11 after ingestion of an infected blood-meal

Days post-infection	Total no. third-stage larvae	No. (%) third-stage larvae found in		
		Head	Thorax	Abdomen
6–8	4	1 (25)	3 (75)	0 (0)
9–11	37	20 (54)	17 (46)	0 (0)
Total	41	21 (51)	20 (49)	0 (0)

The average length of four third-stage larvae measured was 474.2 μm (range, 462.7–498.9 μm), and the width, 18.3 μm (range, 16.6–19.2 μm). The relatively small size of the third-stage larvae is probably attributed to the high temperature under which larval development took place, and the fixation of samples.

The average number of larvae per positive fly examined on days 6–11 was 3.4 with the range of 1–19. And, those of third-stage larvae in any part of the body and also in the head per positive fly were 2.9 (range, 1–11) and 3.3 (range, 1–7), respectively (Table 1). These numbers were slightly smaller than the corresponding one observed for microfilarial intake (average, 4.5 and range, 1–10) (Table 1).

The daily probabilities of survival in the group of flies which fed on the infected volunteer are shown in Figure 1, together with those in the control group. Within 24 hours of feeding, no marked mortality was observed in both groups. This is probably due to the scantiness of ingested microfilariae. Moreover, there was no difference in the daily survival rates during days 2–11 between the two groups (Figure 1).

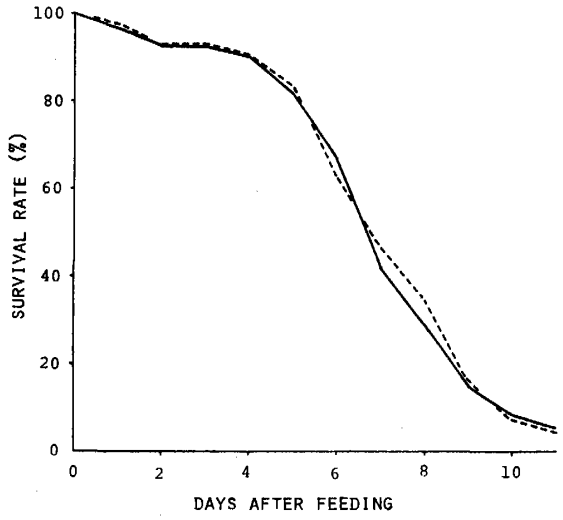

Figure 1 Survivorship curves of *Simulium metallicum* which fed on the infected (solid line) and control (dotted line) persons, respectively, and were kept at a temperature varying between 22°C and 28°C.

Discussion

Duke (1970) has pointed out that *S. metallicum* in Venezuela differently behaves as a vector of onchocerciasis, as compared with the same species in Guatemala. In fact, the apparent difference has been observed with regard to the preimaginal site and the host preference. In Venezuela, *S. metallicum* breeds in small streams, and shows a strong anthropophily (Lewis and Ibáñez de Aldecoa, 1962), while this species in Guatemala utilizes a wide range of watercourses as a larval site, and prefers animals to man for feeding (Dalmat, 1955). Further, there seems to be a slight difference in biting preference for the site on human body. According to Duke (1970), *S. metallicum* in Venezuela has a strong predilection for the lower parts of the body (96.5% of the total catches), whereas the same species in Guatemala bites the upper parts as well as the lower ones (26% vs. 74%). However, it remained unverified whether there is any difference in susceptibility of this blackfly species to infection with the indigenous strain of *O. volvulus* between the two countries. This was mainly due to the lack of data on the degree of susceptibility of *S. metallicum* in Venezuela, although experimental infection studies have been made by Duke (1970) and also by Ramírez Pérez *et al.* (1976).

In this context, the present experiment was performed, and it demonstrated that the development of *O. volvulus* larvae was asynchronous, as reported in *S. metallicum* in Guatemala (De León and Duke, 1966; Collins, 1979; Ito *et al.*, 1980). Namely, an overall proportion of the third-stage larvae among all larvae recovered throughout days 6–11 was 35 per cent, the remaining larvae being still in earlier stages (Table 2).

It is, however, noteworthy that although retarded in development, 69 per cent of the larvae found on days 9–11 had already completed their maturation to the infective stage (Table 2). The proportion of third-stage larvae among all larvae

recovered on days 8–10 in the Guatemalan *S. metallicum* was 26.3 per cent by Collins (1979) and 53.6 or 64.0 per cent by Ito *et al.* (1980). The low rate reported by the former author was probably due to the low temperature condition fluctuating between 16°C and 25.5°C under which flies were maintained. However, it is interesting that even the relatively high rates reported by Ito *et al.* (1980) are slightly lower than 69 per cent observed in the present study, in spite of the fact that the maintaining temperature (constant 25°C) used by Ito *et al.* (1980) was more stable and suitable for larval development than the condition used in our experiment. Moreover, the rate of third-stage larvae moving to the head (29.5%) remarked by Ito *et al.* (1980) seemed to be very low, as compared with 54 per cent in our result (Table 3), suggesting that the probability of infective larvae eventually invading man is higher with the Venezuelan *S. metallicum*, than with the Guatemalan one. When the difference in the temperature condition in experiments is taken into account, this could be a reflection of the difference in degree of susceptibility of *S. metallicum* to the indigenous strain of *O. volvulus* between the two areas.

Further, abnormal and deformed larvae were rarely seen in the present experiment. This is in marked contrast to the observations made in the Guatemalan *S. metallicum*, in which such larvae were frequently encountered (De León and Duke, 1966; Collins, 1979; Ito *et al.*, 1980).

In our study, the decrease in number of the microfilariae ingested by *S. metallicum* during the migratory and developmental phases was inconspicuous. The average number of third-stage larvae in any part of the body and in the head per positive fly (i.e. each 2.9 and 3.3) were only slightly smaller than that of microfilariae ingested (i.e. 4.5). This may reveal a good adaptation of the parasite to the Venezuelan vector.

It is suspected that flies which had ingested more microfilariae may have died earlier than the other, as the infection rate, as well as the average number of larvae per positive fly during days 1–5 (Table 1), was somewhat higher than those attained during later periods. However, it seems to be the rule that survival of female *S. metallicum* is scarcely affected by the presence of the parasite therein, if small in number. As many as 11 third-stage larvae were found in one fly examined on day 10.

From the findings mentioned above, it is likely that apart from the difference in the host feeding preference and the preimaginal habitat, the Venezuelan *S. metallicum* somewhat differs from the Guatemalan one even in susceptibility to the indigenous strain of *O. volvulus*.

On the other hand, Tidwell *et al.* (1980), from the experimental infection study in Colombia, reported that out of 206 larvae in *S. metallicum* examined on days 7–13, 25 per cent were abnormal, 69 per cent had not developed beyond the first stage and only 10 per cent had reached the third stage. Engorged flies were kept at a temperature varying between 22°C and 27°C, being almost the same condition as used in our experiment. Their results suggest that the susceptibility of *S. metallicum* in Colombia to the indigenous strain of *O. volvulus* remarkably differs from that of the same species in Venezuela.

In conclusion, the relatively high susceptibility remarked in our experiment, in combination with the predominancy and the high anthropophily (Lewis and

Ibáñez de Aldecoa, 1962), may explain why *S. metallicum* plays a major role in transmission of onchocerciasis in the northern areas of Venezuela, unlike in Guatemala and Colombia.

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ベネズエラにおける *Simulium metallicum* の *Onchocerca volvulus* に対する感受性について[1]

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ベネズエラ北部のオンコセルカ症伝搬ブユ種である *Simulium metallicum* の *Onchocerca volvulus* に対する感受性の程度を実験感染により検討した。実験には，*O. volvulus* ミクロフィラリア保有者の脚から充分吸血した野外の雌成虫を用いた。これらの吸血個体を 22–28°C の温度条件下で個別に飼育し，毎日死亡した個体を解剖することにより幼虫の発育を調べた。その結果，吸血後5日と6日の間に死亡したブユから最初の第 III 期幼虫が見いだされた。幼虫発育は不揃いであったが異常形の幼虫は見られなかった。ブユ体内で見いだされた幼虫のうち第 III 期幼虫の占める割合は吸血後6–8日に剖検したブユ体内で6％であったが，9–11日に調べたブユでは69％と日数の経過とともに増加した。同期間におけるブユの頭部に見いだされた第 III 期幼虫の割合も25％から54％へ上昇した。すなわち，*S. metallicum* 体内での *O. volvulus* 幼虫の発育は不揃いであるが，ブユが長く生存すれば，とりこまれたミクロフィラリアの多くは第 III 期幼虫まで発育し，人へ感染する機会が多いことが示された。これらの結果と既に報告されているグアテマラとコロンビアでの実験感染の結果を比較すると，ベネズエラの *S. metallicum* は上記2国の同一ブユ種より，*O. volvulus* に対して高い感受性をもっていることが示唆された。

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(次号に掲載)

シンポジウム

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1 国内外におけるマラリアの現状と対策

大鶴 正満 （琉球大・医・寄生虫）

周知のように，第2次大戦を契機として出現したDDTの残効性を利用したマラリア根絶対画も，その効果に限界のあることが明らかになり，1967年ごろから方向転換を余儀なくされるに至った。そして近年，世界各地で再びマラリアの増加傾向がみられ，現在も世界人口のほぼ半数に当たる約21億人がマラリア流行地に住み，毎年1.5億の人々が感染しており，マラリアは今日なお人類をおびやかす重大な疾病であることに変りがない。

昨年の本総会では「マラリアの基礎的研究」シンポジウムが持たれた。引き続き本年は視野を変え，「国内外におけるマラリアの現状と対策」としてマラリアの主として疫学的方面のシンポジウムが企画されることになった。それほどマラリアの問題は，わが国内外でも重要視されねばならない事情にあることを示していると解すべきであろう。

本シンポジウムでは，国外のマラリア事情として鈴木守，神原廣二，栗原毅の3氏により，それぞれスーダン，インドネシア，タイにおける現状が報告される。そこでは熱帯地方における農業開発と寄生虫病，2国間協力によるマラリア対策の現状，多様化したマラリア対策のあり方等が取り上げられる。国内事情については大友弘士，長谷川英男の2氏により，それぞれ増加傾向にあるわが国の輸入マラリア，再び土着の危険性を持つに至った亜熱帯環境下の沖縄における現状等が報告される。

本総会では，以上の演者による発表のほかに，国内のマラリア症例，診断法，更には海外におけるマラリアの疫学的調査等に関する7演題がみられる。本シンポジストはもとより，これらの発表者も加わり，活発な討論が行われることを期待したい。

2 スーダンの新しいマラリア対策

鈴木 守 （群馬大・医・寄生虫）

スーダンは東アフリカ北緯4°-22°に位置し，エジプト（北）エチオピア（東），ケニア（南），中央アフリカ（西）など8か国に囲まれた平坦な国である。国土は日本の約7倍の面積をもって構成されている。西側を白ナイルが，東側を青ナイルが南より北に流れ，首都カルトゥームで合流する。総人口は，1,830万人（1981）であり，農業を主要産業としている。青ナイルと白ナイルにはさまれた三角州地帯はゲジラ（島の意）地方と呼ばれ，大規模な灌漑用水路がはりめぐらされている。ここに，綿花，麦，さとうきび等を主要産物とする一大農業地帯が展開されている。スーダンが将来の食糧庫として期待されているゆえんである。スーダンの灌漑用水路は，スーダンの主要産業の農業にとって最も基本となる農業用水をゆきわたらせたが，同時に水に由来する疾病，すなわち住血吸虫症，下痢性疾患，そしてマラリアをはびこらせる結果にもつながった。

マラリアの突発性流行は1974-75年にかけて，最も中心的な農業地帯であるゲジラ地方におこり，たまたま時期が綿の収穫期と一致したため，スーダンの財政上に大きな影響としてあらわれた。この事件は，同国の農業政策推進上，保健問題，特にマラリア対策を同時にすすめることが不可欠であることを示した極めて重要な事件であった。この事件を契機としてマラリア対策が一段と強化されたが，同地方のハマダラカ，*Anopheles gambiae arabiensis* はすでにDDT耐性を獲得していたため，代って有機リン系殺虫剤，マラチオンが導入されて室内残留散布が行われた。その結果，マラリアの流行は急激におさまり，コントロール計画は成功した。しかし，マラチオン散布5年目にし

てマラチオンに対する耐性を獲得した *A. gambiae arabiensis* が出現し，コントロール計画に破綻のきざしが具体的にみられるようになった。スーダン厚生省はここで独自に予備テストを行い，別の有機リン系殺虫剤，フェニトロチオンが，マラチオン耐性を獲得したハマダラカにも極めて有効に作用することをみいだし，フェニトロチオン散布の方針を打ちだした。日本政府はこうした実情にかんがみて，1980年，180万ドル相当の無償援助を行うことを決定し，フェニトロチオン，抗マラリア剤，車輌，顕微鏡等を送付した。さらに日本政府は1982年，スーダンのマラリア対策に対して第2次援助を行った。この様な努力のつみ重ねにより，ゲジラ地方のマラリア発生数は1％以下におさえられている。特に集中的に対策をおしすすめたモデル地域すなわち“Study Zone”においては，過半数の村落の小児原虫保有率は0％となり，平均0.41％という成績が示されている（1981年度）。

しかし，このままフェニトロチオンを散布しつづけるなら，早晩フェニトロチオン耐性ハマダラカが発生し拡散してしまうはずである。WHOはこうした局面に立って，Primary Health Careを中軸とした総合的な抗マラリアキャンペーンを展開し，殺虫剤の使用をできるだけ早く切りあげることを推奨している。これに対し，スーダン側関係者は，日本側助言者と協議し，マラリア発生率が激減したゲジラ地方の殺虫剤散布は早々に切り上げ，これに代ってゲジラ地方を囲むカルトゥーム，白ナイル，青ナイルの各州の殺虫剤散布を強化させる方針，すなわち“Barrier Zone”を設定し，それを拡大させていく方針をうちだしている。“Barrier Zone”の内側，すなわちマラリアの発生・流行が低下した地域は，さらに詳細・綿密な調査をすすめ，潜在的に残っている流行の発生源を追いつめ，ここを徹底してたたく方針が考えられる。こうした進め方が可能なことは，一部，ハイティで行われた血清疫学の結果からも示唆されている。いずれにせよ，スーダンのマラリア対策は，対策方針が行詰りをみせている現在のマラリア計画の状況に新しい1つの方向を示すものとして注目されてよいだろう。

3　インドネシアのマラリア事情と対策

神原　廣二　　（阪大・微研・原虫）

現在インドネシアにおけるマラリア対策は全地域にまで及ぼす事が不可能なため　1）ジャワ，バリ島におけるendemic focusの減少，2）ジャワ，バリ島以外では移民地域，経済発展に重要な地域及び国境地域を重点的に対策を行う，というものである。ジャワ，バリ島で最も問題となっているのは中央ジャワの一部の県に集中するendemic malariaで，媒介蚊 *Anopheles aconitus* がすでにDDT耐性であり，しかも水田繁殖蚊であるため灌漑用水の発達により年中変わらず発生するなどの条件が重なり防圧対策が非常に困難になってきている。又 *Plasmodium falciparum* のクロロキン抵抗性に関しては最近まで患者治療によるWHO in-vivo methodが用いられていたため，イリアン・ジャヤ，東カリマンタン，南スマトラにおいての報告のみであったが，Rieckmanによるin-vitroのmicro-testが用いられて以来，全国の各地域から抵抗性株が報告されている。

さて1979年以来，イ・日協力で始まった北スマトラ地域保健対策プロジェクトの一部としてマラリア・コントロール計画が始められ，プロジェクト地域とされたアサハン県の3郡におけるマラリアは *An. sundaicus* によって媒介される事，そこでの流行地域は海岸に面した地域であり，その範囲も1カ村に及ぶ大きさもない数個の部落に限られたものである事，マラリアの伝播は乾期に入り次第に活発となり，雨期には殆ど伝播のない事などが明らかにされた。これらのデータを基にDDT散布が行われたが，対蚊，対患者発生ともに見るべき効果は認められなかった。これは散布のcoverage rateの悪さにも基因すると考えられその後2回のDDT散布が加えられたが，同様の結果を得た。このため現在ではDDTに変わるコントロール方法を検討するため，対幼虫対策を中心とした計画が施行中である。また対人についてもクロロキン抵抗性の確認，それに変わる薬剤の検討などが急がれている。

4 タイ国のマラリア伝播と対策

栗原　毅　（帝京大・医・寄生虫）

多くのアジア諸国と同様，タイ国のマラリアは，かつて国民の死因の第1位であり年発生率（API）が300/1,000人を示した第2次大戦までの時代と，戦後のマラリア対策により2/1,000人にまで低下した60年代を経て，現在のコントロール計画に至っている。しかし，問題点が少なからず，計画遂行は必ずしも円滑ではない。たとえば，国民の現金収入を求めての居住民移動や山村への移動拡大，国境付近地域での難民流入といった社会情況の変動，あるいはマラリア治療薬剤に対する耐性の出現と普及，熟練した技術者の不足，器材やガソリン等の経費値上げに伴う対策予算の不足，などが障害となっている。

コントロール計画の実施対象地域は，先の根絶計画でカバーできなかった国境周辺部が主体で，国民人口の20%が居住している。主な作業は，DDT 屋内残留噴霧による伝播の阻止と，患者探索・治療の2点である。残留噴霧はDDT 2 g/m²によるが，近年は民衆のDDT に対する慣れ，飽きに由来する散布忌避の増加，その為の効力低下，加えてDDT の入手難などが原因して卓効をしめすケースが少ない。患者探索は，アメリカの援助とボランティアなど一般住民の協力も得て積極的に進められ，API の信頼度を高めている。しかし，残留噴霧に代わる適当な対策作業は目下のところない。事態の改善を意図して，日本政府の協力でDDT に代えフェニトロチオンの導入が一部地域で実施されている。

今後の研究課題は多い。この地域の媒介蚊の生態的知見充足は，その1つの急務である。コントロール地区の2種の主媒介蚊の中で，*Anopheles minimus* が屋外休止習性を獲得したといわれ，もう一方の *An. dirus* は，従来から屋外休止性があるといわれてきた。両者に対して屋内残留噴霧が，どのように作用しているのか，明らかになっていない。またコントロール作業が経時的に進捗し，API を低下させた土地では，残留噴霧を中止し，その後はベクター増加を防止するための対策がとられねばならない。幼虫発生源処理は，手近にアプローチしうる対策であろうが，どのような技術，組織で推進するか。その実施向上には，地域住民の組織的な参加協力が不可欠であろう。このような，残留噴霧終了後の対策立案は，多くの方法が提唱されてはいても，具体性に乏しい現状である。

現在作業中の土地も，また対策が一段落した土地でも，突然に起こりうるであろう再流行に対する監視は不可欠である。蚊群が示す，流行が強まる際にみせる兆候については，既に明らかであり，媒介能の大小についても算出が可能である。しかし，目下は，広域で監視作業をする組織を欠き，またフィールドでの試みも充分ではない。

5 マラリア：わが国における現状と対策

大友　弘士　（岐阜大・医・寄生虫）

演者らは1972-1981年のマラリア発生状況を調査し，わが国における輸入マラリアの実態の究明を試みた。その結果，697例の患者を検出したが，これは同期間内に厚生省に届け出された349例をはるかに越えるものであった。これらの虫種は *P. vivax* (61.0%) が最も多く，ついで *P. falciparum* (28.4%), *P. ovale* (2.2%), *P. malariae* (2.0%) となり，3.4% が不明であった。虫種と感染地との関連をみると，アジアとオセアニアで感染した467例の72.2%が *P. vivax*, 20.3%が *P. falciparum* であったのに対し，アフリカで感染した180例のそれは54.4%が *P. falciparum*, 28.9%が *P. vivax*, 8.3%が *P. ovale* であった。また，南米（12例）と東地中海地区での感染者の虫種は大部分が *P. vivax* であった。さらに，国内感染が5例検出され，輸入症例からの偶発的な血液接種による二次感染と推定された患者（*P. falciparum*）とベトナム人の母親から娩出された新生児（*P. vivax*）を除く，3例（*P. vivax*）の感染経路は不明であった。なお，これらの患者の79.1%が日本人，20.1%が日本で発症した外国人であった。一方，現在マラリアの流行がみられないわが国では，一般医家はマラリアに対する臨床経験に乏しく，その対応に適切を欠く場合も少なくないのが実情である。そのため，熱帯熱マラリア患者の死亡例が少なくなく，三日

熱マラリアでは治療後の再発率が高いことが指摘されている。したがって，マラリアの診断に習熟するとともに，本症，特に熱帯熱マラリアの特異療法と臨床的管理の方策を早期に確立することがマラリア対策の緊急の課題であるが，それには効果的で安全性の高い抗マラリア薬の確保を国家的なレベルで配意することも必要であろう。

6 沖縄県における最近のマラリア事情

長谷川英男　（琉球大・医・寄生虫）

最近の沖縄県におけるマラリアの発生状況，対策等について報告した。

1970年から1983年7月の間に48例（日本人40例，外国人8例）がマラリアと診断された（安里，長谷川，1981；安里による資料）。虫種が同定された43例中では三日熱が35例で最も多く，次いで熱帯熱7例，四日熱1例で，卵型マラリア及び混合感染はみられなかった。なお，これらの外に米軍関係者で1977年2月から1981年5月の間に熱帯熱36例，三日熱6例，両種混合3例の計45例のマラリア発生が知られている。

患者（米軍関係者を除く）48名は全て男子で，年齢は0歳から58歳に及んでいるが，42名（88%）は21—50歳の間にある。日本人患者40名は2名を除いて全て遠洋カツオ漁業従事者であり，居住地は36名が宮古群島，2名が沖縄本島，1名が石垣島であった（1名は不明）。また外国人患者8名のうち3名はベトナム難民であり，その収容施設は沖縄本島本部にある。他の外国人患者はいずれも旅行者ないし一時的滞在者である。

日本人患者の38名（95%）はパプア・ニューギニア地域で感染を受けており，外国人患者は出身国あるいは沖縄到着前の渡航先で感染を受けている。なおベトナム難民新生児に輸血によるとみられる症例があった。

治療にはクロロキン，クロロキン・プリマキン合剤，新MP錠，キニーネ等が用いられている。予後は死亡1例（熱帯熱），再発1例（三日熱）が知られているが，他に再発・再燃の例は報告されていない。

沖縄県ではマラリア媒介蚊は周年繁殖しており，最近（1981-1982年）のライトトラップによる捕集成績では，石垣市名蔵で捕集蚊総数12,716個体中，シナハマダラカ（*A. s.*）1,651個体，コガタハマダラカ（*A. m.*）156個体；宮古島仲里で総数1,973個体中，*A. s.* 170個体，*A. m.* 387個体；沖縄本島本部で総数329個体中，*A. s.* 4個体が得られている（宮城，当間，岸本，1983）。

県では，県内医療機関でマラリア発生があった場合，直ちに届け出をさせると共に，血液塗抹標本の提出を求め，虫種の同定を行って患者の治療等に指導・助言を与えている。また媒介蚊の調査も継続して行っている。一方，遠洋漁業会社に対する予防内服の指導も行っているが，その服用は徹底していないようである。

以上のように，沖縄県では輸入マラリアの発生が絶えず，媒介蚊も依然として多数繁殖している。従って，再びマラリアが土着化する危険性が大きい。これを防ぐためには，マラリア流行地からの帰国者，入国者に対する，十分なマラリア監視体制の整備が望まれる。

II　毒蛇咬傷における蛇毒の毒性因子の役割

1　毒蛇咬症における蛇毒の毒性因子の役割

沢井　芳男　　（日本蛇族学術研）

我々は第24回日本熱帯医学会のラウンドテーブルディスカッションでハブ咬症の局所病変におけるハブ毒の毒性因子について討論を行ったが，今回は主にアジア地域の毒蛇咬症の病因をテーマとしてそれぞれの蛇毒の毒性因子について討論を試みる。また本シンポジウムではナミヘビ科のヘビであるヤマカガシ咬症の病因についてもふれる。

一般に毒蛇はコブラ科，クサリヘビ科，ウミヘビ科のヘビ類に分類されているが，これ等のヘビ類の蛇毒はハブ毒等で代表される出血毒及びコブラ毒の神経-筋肉接合部に postsynaptic に働く神経毒に大別されている。しかし個々の毒蛇咬症をみると同じ科に属するヘビでもそれぞれ特徴のある症状を呈するので，それ等の症状から咬症を鑑別することができる場合が多い。例えば筋壊死，血液凝固異常，腎障害，筋肉痛，ミオグロビン尿等がそれである。

またそれを裏づけるように個々のヘビ毒の構成をたどっていくと上記の特性の他にさらに個々のヘビ毒を特徴づけるような因子によって修飾されていることがわかる。すなわち壊死，血液凝固，presynaptic に働く神経毒因子，カルジオトキシン，ミオグロビン尿に関与するホスホリパーゼ A_2 等である。

そこで本シンポジウムは，これまで明らかにされつつある毒蛇咬症の病因に関与する蛇毒の毒性因子を中心に，討論が進められる。

2　毒蛇咬症の病理

本間　学　　（群馬大・医・病理）

毒蛇咬症は局所変化と全身性変化に分けられる。前者では広範にわたる組織壊死とその後遺症が，後者では全身性出血傾向，急性腎不全，二次性ショックが問題点としてあげられる。

局所変化は浮腫，出血，組織壊死で代表されるが，蛇毒によってその程度は多少異なる。一般にコブラ科の毒は浮腫と組織壊死を示し，出血を惹起しない。クサリヘビ科やマムシ科の毒は浮腫，出血と組織壊死を招く。出血は毛細血管を中心とする微小血管の破綻によるもので，毒の拡散とともに遠心性に拡大する。コブラ毒でも毛細血管の内皮の変性，壊死が生ずるが，出血毒と異なり基底膜の破綻がなく，また血流が途絶している。血流の存在と基底膜の破綻が出血には必須の条件であることを示唆している。毒による筋壊死はクサリヘビ科，マムシ科の毒だけでなく，コブラ科の毒でも惹起される。形態学的には凝固型と融解型の２型に大別される。凝固型はプロティナーゼ，出血因子で生じ，易熱性である。融解型はホスホリパーゼＡで生じ，耐熱性である。一般に融解壊死を主体とする筋壊死は抗血清治療に抵抗性である。下腿，前腕，手指などの咬傷では，筋束の広範な壊死と機能障害を見ることがある。これらの症例に共通する所見は，解剖学的に狭い区画に位置し，筋膜や腱鞘の発達した筋束に毒が注入されたと推定されることである。そのため出血，血漿性滲出液の貯溜が筋束内組織圧の異常な上昇を招き，領域動・静脈の壊死性及び閉塞性変化とともに高度の阻血を起こし，毒作用と相乗して治癒の遷延と広範な筋壊死を起こすと考えられる。

咬傷局所から血中に吸収される毒成分は各蛇毒特有の作用を全身性に表現する。コブラ科の毒は神経毒として知られ，神経・筋接合部に作用し，呼吸運動麻痺と循環不全を招く。形態学的に変化に乏しく，わずかに実質臓器のうっ血が見られるのみである。クサリヘビ科のラッセルならびにエキスクサリヘビ，ナミヘビ科のヤマカガシなどのように強い血液凝固因子をもつ毒は全身性の出血傾向と急性腎不全を惹起する。毒を動物の筋肉内に注射すると，肝，腎，肺などの細血管にフィブリン塊が生じ，時間の経過とともに腎糸球体に広範にわたる血栓形成を起こす。しばしば近位尿細管の巣状壊死を伴い，肺や心には出血をみる。血

小板の減少，フィブリノーゲンの低下，全血凝固時間の延長があり，腎を中心とする播種性血管内凝固症候群(DIC)を起こしている。ハブやマムシ毒ではDICの所見はなく，実質臓器に全身性低酸素血症にみられる変性と散在性壊死が認められ，まれにショック腎の所見を呈している。クサリヘビ科とマムシ科の蛇毒による全身性変化は，1つはDICを，他は二次性ショックを基本とした変化に要約出来る。

3 神経毒因子

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コブラ，アマガサヘビ及びタイガースネーク毒の主要致死因子は神経毒であり，これらの毒素が神経系に作用する部位によってpresynapticとpostsynaptic トキシンに大別される。

Postsynaptic トキシン：台湾コブラ毒から分離，結晶化された結晶毒蛋白Cobrotoxinは，分子量約7,000の強塩基性のポリペプチドであり，脊椎動物の骨格筋と神経の接合部にあるAcetylcholine receptor (AchR) と特異的に結合し，神経末端より放出されるAcetylcholine (Ach) がAchRに作用するのを防ぐ。その結果，神経筋シナプスの伝達は停止する。アマガサヘビ毒より分離されたα-Bungarotoxin (α-Bgt) もCobrotoxinと同じ様に，postsynaptic トキシンに属し，シナプス後部細胞膜にあるAchRと特異的に結合する。

Presynaptic トキシン：一方，同じアマガサヘビ毒から分離されたβ-Bungarotoxins (β-Bgt) はPresynaptic トキシンに属し，神経筋接合部において，前シナプス膜に作用して，Achの放出を阻止する。β-Bgtは分子量21,500，等電点9.5の蛋白質であり，S-S 結合で連結された2本のポリペプチド鎖（120残基のA鎖と60残基のB鎖）から成り，Phospholipase A_2 (PLA_2) 活性を有している。

オーストラリア産のタイガースネーク毒から分離されたpresynaptic トキシンNotexinも，β-Bgtと同じ様にPLA_2活性をもつが，分子量13,600の1本鎖の蛋白質である。ブラジルのガラガラ蛇毒から分離されたCrotoxinとタイパン蛇毒からのTaipoxinもpresynaptic トキシンとしてよく知られており，PLA_2活性をもつ。そのSubunitはβ-BgtのA鎖及びNotexinと同じ様にPLA_2構造をもち，すでに一次構造が決定されている蛇毒のPLA_2とよく似ている。

これらpresynaptic トキシンのPLA_2活性には，他のPLA_2と同じ様に，Ca^{2+}が必要であり，p-Bromophenacyl bromide (BPB) によって活性中心にあるHis-48が特異的に修飾されると失活する。このBPBによる化学修飾やSr^{2+}を用いた実験の結果は，β-Bgtによるシナプス伝達阻止のためにはPLA_2活性が必要であることを示唆している。しかしながら，Neurotoxic PLA_2であるβ-Bgtを基質存在の下に，Ethoxyformic anhydrideで修飾すると，Non-neurotoxic PLA_2に変る事実と，KCNOによるLys-残基の修飾によって，酵素活性がほとんど変らないにもかかわらず，毒性と薬理作用が著しく低下または消失する事実は，毒性と各種薬理作用の発現とPLA_2活性の間には必ずしも直接関係がなく，PLA_2には少なくとも2つの活性部位，すなわち酵素活性中心と毒性及び薬理作用と関係のあるNon-enzymatic siteのある事が示唆される。

Cobrotoxin の構造と生物活性：現在postsynaptic トキシンで，アミノ酸配列の決定されたものは80以上を数えるに至り，その構造から短鎖型と長鎖型トキシンに分けられる。アミノ酸62個から成るCobrotoxinは短鎖トキシンに属し，長鎖トキシンα-Bgtは74個のアミノ酸から成る。α-BgtはCobrotoxinと同じ位置に4個のS-S結合を共有する他に，もう一つのS-S結合をもち，余分の小ループをなす。このS-S結合は毒蛋白分子の表面にexposeして居るので，選択的還元されても構造には変化をきたさない。またC末端に計7つのアミノ酸残基から成る尾がついているが，これも神経毒作用と直接関係がない。

Cobrotoxinはその4個のS-S結合を還元すれば構造の変化を起こし，毒性，抗原性を完全に失い，これを空気中で再酸化すれば，SH基の消失

に伴って毒性と抗原性を完全に回復し，ORD 曲線も原毒のそれと一致した。Tyr-25 がニトロ化されると，毒蛋白の構造に変化が起こり，毒性と抗原性を完全に失う。このことは S-S 結合と分子内に埋れた Tyr-25 は Cobrotoxin の生物活性の発現に必須な構造を保つに必要である事を示す。

またリジン及びアルギニン残基の選択的化学修飾によって，Lys-47 の ε-NH_2 と Arg-33 のグアニド基の陽イオン群が，運動神経終板の AchR の陰イオンの部位と特異的に結合することにより，神経筋伝導を遮断することが認められた。

4 毒蛇咬症と血液毒

岩永　貞昭　　(九大・理・生物)

マムシやハブ，クサリヘビに咬まれた動物の血液が極めて凝固しにくく，また著しい血小板減少のあることは，今世紀の始め頃から知られていた。しかし，当時は止血のしくみはもとより，血液凝固についての知見にも乏しかったため，蛇毒が止血過程のどこに作用するか殆ど不明であった。その後1930年代に入って，Eagle はある種の蛇毒がプロスロンビンの活性化とフィブリン形成を惹起することを明らかにし，一方 Macfarlane らもほぼ同じ頃，*Viper russelli* 毒が血友病患者の止血に有効であることを見出した。蛇毒の血液毒成分はこうした実用面での開発とあいまって，医学領域で次第に注目されてきたが，その作用点が明確になってきたのは近年のことである。今日では，英文表に示す如く，多数の血液毒成分が精製，分離され，それらの生化学的諸性質も明らかにされている。

さて，ヒト血漿中に含まれる凝固因子はすべて不活性前駆体として存在し，ある種の分子集合体を形成している。凝固系がいったん活性化されると，活性型となり機能を発現したあと，すみやかに antithrombin III や α_2-macroglobulin, C1′inactivator などの血中プロテアーゼインヒビターによって阻害される。また各凝固因子の活性化には，Ca^{2+} のほか血漿中のプロテインコファクター（VIII 因子や V 因子など）やリン脂質が必須で，それらの共存下で活性化速度は著しく亢進される。また，活性型プロテインCによるプロテインコファクターの分解を介して，IX 因子や X 因子の活性化速度が調節されている。このように凝固因子の活性化は凝固カスケード系の中で，相互に制御されつつ恒常性を保っている。

一方，蛇毒に含まれる凝固因子（表のスロンビン様酵素，プロスロンビン活性化酵素，X 因子活性化酵素など）はすべて活性型であり，かつ血漿のプロテアーゼインヒビターにより阻害されない。これは血中の凝固因子がセリンプロテアーゼであるのに対し，蛇毒の凝固因子は金属プロテアーゼの一種であることに基づく。また，蛇毒凝固因子の多くは血漿中のプロテインコファクターによってその活性が増強されることもない。従って，ひとたび蛇毒凝固因子が血中に入ると，血液本来のもつ止血制御系とは無関係に，一方的に凝固因子を活性化ないし不活性化することになる。これは毒ヘビ側からみると合理的かつ攻撃的な作用ともいえる。最近，この一方的ともいえる蛇毒凝固因子の作用機構が，ヒトの血液凝固系の解明とあいまって一層はっきりしたが，本シンポジウムでは，主に血液の凝固障害を惹起する蛇毒成分に限って，それらの生化学的諸性質および作用点について述べた。

5 毒蛇咬症におけるミオグロビン尿因子と蛇毒のホスホリパーゼA

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ウミヘビ（*Enhydrina schistosa*）の毒では60%を神経毒が占めるにもかかわらず，咬症はミオグロビン尿を呈するのが特徴的であり，myotoxin が単離され塩基性ホスホリパーゼA蛋白であることが知られている。一方，オーストラリア産コブラ科の咬症でもミオグロビン尿を呈することが知られている。著者らは，10種のオーストラリア産蛇毒（*Oxyuranus s. scutellatus*, *Notechis s. scutatus*, *N. ater serventyi*, *N. a. humphreysi*, *Acanthophis antarcticus*, *Austrelaps superba*, *Pseudonaja textilis*, *Pseudechis australis*, *Pseudechis porphyriacus* and *Pseudechis colletti*）を CM-Sephadex C-25, SP-Sephadex C-25 を用いるカラムクロマトグラ

フィーで分画し，皮下注後，ミオグロビン尿を呈する活性を調べた。10種のうち，4種(*A. superba*, *P. australis*, *P. porphyriacus* and *P. colletti*) の蛇毒から4種の単一な画分を得た。これらは何れも中性又は塩基性のホスホリパーゼA蛋白であり120-129個のアミノ酸残基から構成されており，アミノ糖は含んでいない（分子量は13,400-14,200)。これらの酵素の LD_{50} は4.3-7.7 mg/kg (s. c.) であり，ミオグロビン尿は 0.5-5.0 mg/kg (s. c.) で認められた。myonecrosis の臨床的指標として creatine kinase (CK) の活性増加が知られているが，*P. colletti* の fraction II では，ミオグロビン尿を伴って dose dependent な CK の活性増加が認められた。アミノ酸組成上では，*E. schistosa* の myotoxin, presynaptic toxin の notexin と相同性が高い。このことから，アミノ酸配列の相同性も高いものと考えられる。

ホスホリパーゼA活性をもつか，又はそのサブユニット構造を保有している蛇毒成分は，ミオグロビン尿因子の他に，presynaptic toxin, cardiotoxin 及びその他の toxic phospholipase が知られている。これらの蛋白のアミノ酸配列は互いに相同性が高く，殊に His 48 周辺の酵素的活性部位及び界面認識部位と呼ばれるN末端部分の構造の相同性は極めて高い。これらの酵素的活性部位と毒性，薬理的作用との関係は未だに不明な点が多いが，種々の修飾反応の結果から，酵素活性部位の他に，毒性，薬理作用に必要な non-enzymatic site が存在するとされている。又，N末端部分を含み約20残基から構成される界面認識部位のアミノ酸置換が多く認められることから，組織中の基質に対する特異性や組織への親和性の違いが種々の作用の原因と考えられる。

グループ報告

タイ国北部における日本脳炎のウイルス学的疫学的調査

1 1982年タイ国チェンマイ地区における脳炎のウイルス学的疫学的調査—計画の概要，ウイルス分離と免疫酵素測定法(ELISA)—

五十嵐　章
（長崎大・熱帯医研・ウイルス）
緒方　隆幸
（予研・ウイルスリケッチア）
藤田　宣哉　（神戸大・医・微生物）
福永　利彦　（阪大・微研・防疫）
森　章夫　（長崎大・医・医動物）
宇塚　良夫(長崎大・熱帯医研・臨床)

1982年の7月と8月にタイ国北部チェンマイ地区における脳炎の現状を知る目的で，患者及び野外蚊からのウイルス分離，患者の血清反応，健康人及び各種脊椎動物の抗体調査，及び日本脳炎(JE)媒介蚊の調査を実施した。

ウイルス分離はヒトスジシマカ培養細胞クローンC6/36を用い，177検体の入院患者末梢血から11株のデングウイルス（1型8株，2型2株，3型1株）を分離した。このうち9株はデング出血熱(DHF)患者から分離されたが，1型の1株は脳炎患者，2型の1株は髄膜炎患者から分離された。JEウイルスは3検体の死亡脳炎患者脳のうち1検体から分離されたが，野外採集したコガタアカイエカ，*Culex geliclus*, *Cx. fuscocephala* 15,513匹125プールからは分離されなかった。

患者の血清反応はJEとデングの交差反応のため血球凝集抑制反応の結果は必ずしも明確でなく診断確率も高くなかった。JEに対するIgM-ELISAは脳炎患者55例中27例に陽性であったが，79例のDHF患者はすべて陰性であった。健康人の血清疫学調査でJEに対するIgM-ELISA陽性率は5カ所の採血地点によりかなり異なり，調査地域内でJEウイルスの新鮮感染率に差があることが示された。

水田耕作を営まない山地民族が脳炎になる機転は今後調査すべき課題と考えられる。

2 1982年タイ国チェンマイ地区における脳炎のウイルス学的疫学的調査—入院患者の解析—

宇塚　良夫(長崎大・熱帯医研・臨床)
五十嵐　章
（長崎大・熱帯医研・ウイルス）
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藤田　宣哉　（神戸大・医・微生物）
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森　章夫　（長崎大・医・医動物）

目的：1969年以来，脳炎患者の多発しているタイ国チェンマイ地区における脳炎の現状を知る目的で実施した今回の調査の一環として，チェンマイを中心とする14の病院の協力を得て，入院患者における脳炎及び類似疾患の解析を行った。

方法：チェンマイ大学病院小児科を始めとする14病院に1982年7月12日から8月18日の間に入院した，脳炎，髄膜炎，デング出血熱(DHF)を主体とする179名の患者を対象に臨床症状，発症時期，年齢分布，地理的分布を検討した。179名全員から採血を行い，JE及びデングウイルスに対する抗体価測定とアルボウイルス分離を行った(この成績は別に報告する)。また，この間にチェンマイ大学小児科に入院した患者197名全員の疾患を分類し，脳炎及び関連疾患の臨床診断の現状を検討し，また，死亡例3例の脳組織を採取しウイルス分離を試みた。

成績：患者は男102名，女77名，年齢は生後8カ月から81歳にわたる。臨床診断は，脳炎55，髄膜炎8，DHF 77，不明熱11，その他28である。年齢分布は，脳炎1歳—81歳で10—14歳が14名と最も多いが，比較的高齢者も多い。DHFは0歳—

29歳までほぼ均等に分布し，29歳以下で83%を占める。しかしながら，タイ国全体の統計と較べると，両疾患共に，今回の成績は明らかに高齢者側に分布している。現住所から見た患者発生地の分布では，DHF がチェンマイ及びランプーンの都市部に集中的に発生しており，周辺の山岳地域ではほとんど発生を見ていない。一方，脳炎はチェンマイ県中・北部に一様に発生しており，殆ど水田の存在しない山岳・森林地帯にも発生しており，山岳民族にも脳炎患者が数名認められ，内1名の死後脳組織から JE ウイルスが分離された。チェンマイ大学小児科の入院患者197名中，今回の対象疾患は25%を占めているが，DHF でも意識障害，項部硬直を伴う者も認め，我々の血清診断による診断変更もあり，臨床的に区別困難な症例も多数認められた。

3 1982年タイ国チェンマイ地区における脳炎のウイルス学的疫学的調査—血清診断及び血清疫学—

藤田　宣哉　（神戸大・医・微生物）
緒方　隆幸
（予研・ウイルスリケッチア）
福永　利彦　（阪大・微研・防疫）
五十嵐　章
（長崎大・熱帯医研・ウイルス）

1982年 7-8月に チェンマイ市及びその周辺地区における脳炎の現状を知る目的で，患者〔脳炎，デング出血熱 (DHF) など〕 ならびに健康住民の血中抗日本脳炎 (JE), 抗デング (DEN) 赤血球凝集抑制 (HI) 抗体を測定し，血清診断ならびに血清疫学調査を行った。

(1) 患者の血清診断: 対血清 (98例) について脳炎と臨床診断された患者の 11.1% (3/36), DHF と臨床診断された患者の 18.4% (9/49) をそれぞれ JE, DEN と血清学的に診断した。JE 患者の4例を初感染，DHF 患者の6例を再感染と推定した。これに HI 反応上 JE, DEN ウイルス感染の疑のある患者を含めて初感染は7例，再感染は33例で，1969-73年の SEATO 研究所の調査と比べ再感染の増加を認めた。このことはこれら地域の JE, DEN 等ウイルスの散布度が1970年頃に比べ増大の傾向にあると考えられた。臨床的に脳炎と診断された患者の中にわずか6例であるが HI 反応上 DEN ウイルスによる DEN 脳症ではないかと思われる者があった。このうちの1例から DEN-1 ウイルスが分離された。これについては更に検討を要する。

(2) 健康住民の血清疫学調査: 被検血清はチェンマイ盆地に属するサラピー，ドイサケット，メテン，パサン，及びタイ国最北部のメコンバレーに属するフアンの5カ所（いずれも水田耕作地）における健康住民（9区分の年齢階層，合計954名）から濾紙法により集めた。抗体保有率，平均抗体価は共に JE がメテン，パサン，DEN がサラピー，ドイサケット，メテン，パサンで比較的高く，加齢に伴って急上昇した。抗 DEN 抗体保有率は 10-20歳で 80-90% の極値に達した。JE のそれは DEN より遅れて上昇し，特にサラピー，ドイサケットでその傾向が強かった。これに対しフアンは抗体保有率，平均抗体価共，JE, DEN 双方でチェンマイ盆地の4地区に比べ明らかに低く，加齢による上昇も鈍い。また抗 JE, 抗 DEN 双方の抗体陰性者が約半数 (47.4%) と他の4地区 (15-27.3%) に比べ多かった。

まとめ: ① 1970年頃に比べ DEN ウイルスがチェンマイ地域に広く浸透している傾向がうかがえる。特にチェンマイ盆地にこの傾向が強く，メコンバレーに弱い。②調査した地域により差が見られ，メテン，パサンで JE と DEN ウイルス，サラピー，ドイサケットで DEN ウイルスの散布度と住民の免疫度が高い。これに比べフアンのそれは低い。

4 北部タイ・チェンマイ地域住民の日本脳炎及びデングウイルスに対する中和抗体保有状況

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奥野　良信，石嶺　毅，只野　昌之，
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タイ国北部における脳炎のウイルス学的，疫学的諸調査の1つとして，健康人の日本脳炎(JE)ウイルス及びデングウイルス1-4型に対する中和抗体保有状況を調べた。5つの採血地区のうち4つ(Pasang, Sarapee, Doi Saket, Mae Taeng)はチェンマイ盆地内に位置し，Fangはチェンマイ盆地と幾重もの山々でへだてられた別の盆地内にある。しかし，Fangの人々の生活環境並びに生活様式は，チェンマイ盆地内のそれらと差異はない。採血は濾紙法により，9つの年齢階層にわけて行った。得られた総血清サンプル数は，985であった。中和試験は，Peroxidase-anti-peroxidase染色による50%フォーカス減少法によった(Biken J., 21, 137-147, 1978)。

得られた成績を総括すると，(1)チェンマイ盆地内の4地区では，JE及びデングウイルスに対する中和抗体保有状況に著明な差がなく，10-14歳で陽性率は70%あるいはそれ以上に達するが，デングウイルス2型に対する陽性率が他のそれらよりやや低い。(2) Fang盆地においては，JEウイルスに対する中和抗体陽性率が年齢増加と共に上昇するが，チェンマイ盆地内4地区と比較するとその上昇はややにぶい。デングウイルスに対する抗体陽性率は，年齢増加に伴う上昇が認められず，同ウイルスの散布度はチェンマイ盆地内4地区よりはるかに低い。(3)現時点では，Fangを含む全チェンマイ地域において，子供達はまずデングウイルスに暴露され，ついでJEウイルスの感染を受けるというのが，主たるパターンと考えられる。

5 タイ国チェンマイ地区における日本脳炎のウイルス学的疫学調査—諸動物の抗体保有状況—

緒方　隆幸
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五十嵐　章
(長崎大・熱帯医研・ウイルス)
藤田　宣哉　(神戸大・医・微生物)
福永　利彦　(阪大・微研・防疫)

タイ国北部チェンマイ地区では1969年以来日本脳炎の流行が続いている。このような地域における家畜及び脊椎動物の日本脳炎，ならびにデング熱の抗体保有状況を調査することは，それらに対する防疫対策上またウイルスの生態学的見地からも重要である。

我々はチェンマイ大学微生物学教室の協力を得て，チェンマイ地区周辺の豚，軍馬(馬及びラバ)，羊，サル，犬，ニワトリ，ガチョウ，スズメ及びトカゲから採血し，その血清について日本脳炎(主としてJaGAr-01)及びD-1に対するHI抗体の陽性率と抗体価を調べた。

その結果，1) 日本脳炎に対する陽性率はウマ及びラバ(100%)，豚(97%)，羊(93%)，犬(93%)，ガチョウ(17%)，猿(10%)，スズメ(3%)で，ニワトリとトカゲは全て陰性であった。

2) デングに対する陽性率は豚(82%)，犬(80%)，羊(57%)，ラバ(31%)，馬(20%)，ガチョウ(13%)，猿(10%)で，ニワトリとトカゲは全て陰性であった。

3) ウマ及びラバ血清についてJaGAr-01以外の日本脳炎株—中山，P-19Br-82-Thai及び2372-82-Thaiの抗原でも調べたが，中山株に対する抗体価が他の3株より有意に低い値を示した。これは抗原のreactivilityによるものと考える。

4) 各種動物のデングの抗体は日本脳炎の抗体が高いため，Cross reactionによるものであろう。

5) 豚血清のELISAによるIgG抗体は1,600倍以上を陽性にとると74/101=78.2%，またIgM抗体は200倍以上を陽性にとると32/101=31.7%あり，豚が日本脳炎のAmplifierの可能が強いが，他の羊，犬等の血清についてもIgM抗体を測る必要があろう。

6 タイ国チェンマイ地区における *Culex tritaeniorhynchus*, *Culex geridus* と *Culex fuscocephala* の生態とウイルス分離について

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タイ国チェンマイ地区で蚊からの日本脳炎ウイルスの分離を試みた。1982年7月13日から8月12

日の間チェンマイ市の近郊にある4カ所の豚舎にライトトラップを設置し，飛来する蚊を捕獲した。採集された蚊のうち日本脳炎の媒介蚊として知られている *Culex tritaeniorhynchus* 4,934匹，*Cx. gelidus* 7,052匹，*Cx. fuscocephala* 3,526匹から *Aedes albopictus* 培養細胞クローン C6/36 を用いてウイルスの分離を試みた。その結果，*Cx. tritaeniorhynchus* と *Cx. gelidus* からそれぞれ1株の未同定のフラビウイルスが分離されたが日脳ウイルスは分離されなかった。

今回の結果と Gould *et al.* (1974) の成績を考え合せるとチェンマイでの伝播蚊の日脳ウイルス保有率は日本のそれに比べ大変低い。その原因を明らかにするためこれらの蚊の吸血嗜好性を調べたが，ウシやスイギュウがブタよりも好まれているということはなく，ブタも主要な吸血源となっていることが判った。次に *Cx. tritaeniorhynchus* について成虫の生存率を求めたが Gonotrophic cycle を3または4日と仮定した時0.600または0.682となり日本での Buei and Ito (1982) や Wada *et al.* (1969) の結果とほぼ一致している。この結果，チェンマイ地区の日脳媒介蚊のウイルスの低保有率の原因を吸血嗜好性や蚊成虫の生存率に求めることはむずかしいように思われる。日脳媒介蚊のウイルス保有率がこのように低いのにもかかわらず，この地区で日脳が存続しているのは媒介蚊の発生数が大変大きいことによるものであろうと推測される。

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PROCEEDINGS OF XXV ANNUAL MEETING OF JAPANESE SOCIETY OF TROPICAL MEDICINE

17–19 October 1983 Lux Auditorium Toyonaka

CONTENTS

Symposium

Recent malaria status and control at home and abroad, Japan

1 RECENT MALARIA STATUS AND CONTROL AT HOME AND ABROAD, JAPAN

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It has been known since around 1967 that DDT residual spraying which had played a major role in malaria control after the World War II is not helpful enough to cope with malaria. Now about half of the world population reside in endemic areas of malaria and about 150 million people are yearly infected with it.

"Basic research in malaria" symposium was held in XXIV Annual Meeting of this Society, 1982, and successively in this year, "Recent malaria status and control at home and abroad, Japan" symposium was also adopted from the field of malaria. Thus, malaria problem should be considered to be one of the most important diseases at home and abroad, Japan, too.

In this symposium, malaria situation and campaign first in Sudan, Indonesia and Thailand, next in Japan proper and Okinawa prefecture situated at the sub-tropical zone will be delivered one after another.

I, chairman of this symposium, hope that all here present well lively express their ideas and exchange various opinions in this field.

2 A NEW MALARIA CAMPAIGN WORKED IN SUDAN

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(Abstract not received in time)

3 MALARIA ENDEMIC AND ITS CONTROL IN INDONESIA

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In Indonesia, the priority of the Malaria Control Program has been given to all the provinces of Java and Bali, whereas in the outer islands, priority is given only to the socio-economical development areas and transmigration areas. In Java and

Bali, the highest endemic foci remain mainly in Central Java where *Anopheles aconitus*, the main vector mosquito, had already acquired resistance to DDT. Recently, chloroquine resistant falciparum malaria has been detected in various parts of Indonesia by using the in-vitro micro-test of Rieckman.

The International Cooperation Program between Indonesia and Japan on the Project for the Promotion of Health in Northern Sumatra has begun in 1977. The Malaria Control Activity was admitted as one of those in the program. Entomological and epidemiological studies had been carried out since 1979. These studies revealed that malaria endemism was restricted to the coastal areas; *Anopheles sundaicus* was the primary vector; and lastly, that transmission became active in the dry season from January to August. Based on these data, DDT was sprayed in June 1981, December 1981 and May 1982. However, DDT spray showed minimal effect on the reduction of mosquito density and malaria prevalence. Thereafter new methods of malaria control as alternatives to DDT spray are being investigated.

4 MALARIA TRANSMISSION AND CONTROL IN THAILAND

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Thailand has been suffered from numerous malaria infections in many provinces and it is reported that deaths from malaria averaged more than 50,000 annualy, according to the Department of Communicable Disease Control, Ministry of Public Health. In 1949, the government established a malaria control project based on the DDT house spraying, then the control area was expanded progressively. Later the project was reorganized and malaria eradication programme was initiated in 1964. Mosquito vector control was the primary and most effective measure utilized in suppressing the malaria transmission. Accordingly the malaria death rate showed a reduction from 297.1 per 100,000 in 1947 to 10.3 per 100,000 in 1969.

For the past 10 years, malaria control has been the goal in areas of low receptivity as a result of a reduction in the budget with a view to meet the cost of DDT and other technical and social requirement. In the control areas of hilly, wooded and foothill terrain, high malariogenic potential is engendered by the efficient vector mosquitoes, namely *Anopheles minimus* and *An. dirus*. The main anti-vector measure continued to be residual house spray with DDT at the dose of 2 g/m^2 generally twice a year. However, many problems are encountered in the anti-malaria operations in the control area, such as finding of drug resistant strains of *Plasmodium falciparum*; increasing exophily among the vector mosquitoes; refusal of house spraying; and shortage of the operation expense or equipment. In 1981, the malaria division had received Fenitrothion supply and commenced training on the use of this chemical. Then the field trials of the spraying were conducted recently.

Many malarious countries are facing similar situation and associated problems according to various reports such as WHO report (1982). In general, the research

in malaria has become increasingly necessary not only to answer certain basic questions but to solve the technical problems. Field study of vector bionomics, with special references to the resting behaviour, is urgent for further expansion of residual spray operation and also for the development of new anti-adult measures. The withdrawal of the house spray operation should be occurred when the annual parasite incidence descends to a certain level. Thus the study of alternative methods of vector control in order to maintain the vector density at a low level, such as environmental, biological and chemical approach of breeding sources, is also necessary. The sanitary conditions can be greatly improved and breeding habitats reduced if the communities are properly participated to these vector control activities. The enhancement of surveillance operation is also essential and the entomological investigation is an important function of the surveillance. The entomological parameters, such as the vector density and the parous rate, should be recorded as the indicators for the forcasting of malaria outbreaks.

5 MALARIA: PRESENT STATUS AND ITS CONTROL PROBLEMS IN JAPAN

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The authors investigated the incidence of malaria in Japan from 1972 through 1981 for elucidating the actual situation for imported malaria. This investigation detected 697 malaria patients, the number of patients found being much larger than 349 cases as officially reported to the Ministry of Health and Welfare during the same period of time as mentioned above.

As for the causative species, *P. vivax* was most frequently identified (61.0%), followed by *P. falciparum* (28.4%), *P. ovale* (2.2%) and *P. malariae* (2.0%), while it was unknown for 3.4 per cent cases. Regarding the source of infection in relation to the species of parasite, *P. vivax* and *P. falciparum* caused 72.2 and 20.3 per cent, respectively, of 467 malaria cases occurring in Asia and Oceania, while 54.4, 28.9 and 8.3 per cent of 180 cases in Africa were due to *P. falciparum*, *P. vivax* and *P. ovale*, respectively. Most of the cases infected in South America (12 cases) and East Mediterranean area were caused with *P. vivax*.

Out of five cases observed in Japan, one was presumed to be due to the secondary infection with *P. falciparum* through the blood transfusion from a case of imported infection and another with *P. vivax* was a neonate born by a Vietnamese mother, while in the remaining three cases with *P. vivax*, the route of infection was unknown. The patients consisted of Japanese (79.1%) and foreigners (20.1%) whose malarial symptoms started in Japan.

In Japan, where malaria is rarely observed, the doctors in general are poor in clinical experiences concerning this disease and their way of handling patients could often be inappropriate. These facts are pointed out to be responsible for the deaths

of some patients suffering from falciparum malaria and relatively frequent post-therapy relapses of vivax malaria.

For the proper treatment of malaria, it is urgently required, first of all, to promote the proficiency in diagnosis of malaria as well as to institute promptly a specific therapy and clinical management method for malaria, especially for falciparum malaria. It should also be considered at the national administration level to secure the acquisition of effective and safe anti-malarial drugs.

6 RECENT MALARIA SITUATION IN OKINAWA PREFECTURE, JAPAN

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An epidemiological analysis was made on the recent malaria cases in Okinawa Prefecture, Japan.

During the period from January 1971 to July 1983, 48 cases (40 Japanese and 8 foreigners) were diagnosed as malaria (Asato and Hasegawa, 1981; Asato, unpublished data). Among 43 cases on which the malaria species were identified, 35 were benign tertian malaria, 7 malignant tertian and one quartan. Oval malaria and mixed infection were not noticed. Besides these cases, 45 cases (36 malignant tertian, 6 benign tertian and 3 mixed of both) were found in American military servicemen in Okinawa during the period from February 1977 to May 1981 (These American cases are excluded from the following analysis.).

All of 48 patients were males with age ranging from 0 to 58, but 42 (88%) of them were in the range from 21 to 50. Thirty-eight patients of 40 Japanese were the crews of the ocean bonito fisheries working around Papua New Guinea, and were supposed to have acquired infections in this region. Residences of the Japanese patients were Miyako Isls. (36 cases), Okinawa Isl. (two) and Ishigaki Isl. (one) (one unknown). Eight foreigers comprising of three Vietnamese refugees (in Motobu, Okinawa Isl.), two Papua New Guineans, one Korean, one Philippino and one Australian, were considered to be infected in their own countries or on the traveling course before arriving Okinawa, except one Vietnamese newborn infant who was supposed to have acquired infection by the transfusion of blood.

The patients were medicated with chloroquine, mixture of chloroquine and primaquine, mixture of sulfamonomethoxine and pyrimethamine, or quinine. One fetal case (malignant tertian malaria) and one relapse case (benign tertian malaria) have been recorded.

In Okinawa Prefecture, the vectors of malaria parasites are bleeding throughout the year. Light-trap collections carried out in 1981 and 1982 demonstrated 1,651 *Anopheles sinensis* and 156 *A. minimus* among a total of 12,716 mosquitoes collected at Nakura, Ishigaki Isl., 170 *A. sinensis* and 387 *A. minimus* among 1,973 mosquitoes at Nakazato, Miyako Isl., and 4 *A. sinensis* among 329 mosquitoes at Motobu, Okinawa

Isl. (Miyagi *et al.*, 1983).

There is no definite malaria control programme in Okinawa Prefecture at present since the indigenous malaria had been eradicated by 1961. However, the prefectural government has guided the medical facilities in the prefecture to report promptly the malaria cases and to offer the blood-films in order to identify the species and given them an appropriate advice about the treatment. The survey on the vectors has also continued to cover the whole islands. On the other hand, the prefectural government has instructed the ocean bonito fishery companies to prepare the antimalarial drugs. But the adequate prophylactic chemotherapy has not always been followed by the crews.

The presence of imported or induced malaria, including relapse cases and the prevalence of anopheline mosquitoes suggest strongly the possibility to produce endemicity again in Okinawa Prefecture. In order to reduce this risk, a complete malaria surveillance system for all persons from malarious areas is required.

Symposium

Activities of toxic component of venom in snake bite

1 ACTIVITIES OF TOXIC COMPONENT OF VENOM IN SNAKEBITE

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In the occasion of 24th Annual Meeting in 1982, we had a chance to discuss on activities of toxic component of habu venom. In this symposium, we will discuss about venoms of snakes of Southeast Asian origin.

In general, the toxicity of venoms of snakes belonging to viperidae, elapidae and hydrophidae was characterized by their hemorrhagic factors and post-synaptic neurotoxin as shown in habu and cobra. On the other hand, the identification of responsible snake in each bite is possible by the characteristic symptoms such as necrosis, incoagulable blood, renofailure, myalgia, myoglobinuria etc., even though the snakes belong to the same family. This is also supported by the fact that each venom is modified its constitution by those characteristic principles in addition to those above-mentioned factors inducing necrosis, blood coagulation, myoglobinuria, presynaptic neurotoxin, cardiotoxin etc.

Thus the subject to be discussed in this symposium is aimed at the role of the venom factors playing in snakebites.

2 PATHOLOGY OF SNAKEBITE

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(Abstract not received in time)

3 SNAKE NEUROTOXINS

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Neurotoxin are the main toxic proteins of cobras, kraits, tiger snake and sea snake venoms which block neuromuscular transmission and cause death of animals

by respiratory paralysis. Snake neurotoxins are classified into two distinct types, postsynaptic and presynaptic neurotoxins, with respect to the mode of the inhibitory action at the neuromuscular junction.

Postsynaptic neurotoxins block the nicotinic acetylcholin receptor at the motor endplate and produce a nondepolarizing block of neuromuscular transmission, just like d-tubocurarine. Due to their strict specificity, the curarimimetic neurotoxins have proven as important tools for the isolation and investigation of the cholinergic receptor. Presynaptic neurotoxins specifically block neuromuscular transmission by acting on the presynaptic motor nerve terminals and are valuable tools for the investigation of the mechanism of synaptic transmitter release.

Cobrotoxin, isolated in the crystalline state from the venom of Taiwan cobra *Naja naja atra*, blocks the acetylcholine receptors on the postsynaptic membrane and thus blocks the neuromuscular transmission. It is a small basic protein consisting of a single peptide chain of 62 amino acid residues, crosslinked by four disulfide bonds. β-Bungarotoxin isolated from Taiwan banded krait *Bungarus multicinctus* venom also belongs to the postsynaptic neurotoxin, shows a great affinity to the acetylcholine receptors of motor endplates.

Several β-Bungarotoxins, presynaptically acting neurotoxins, have also been isolated from the same krait venom. The toxin completely inhibits the release of acetylcholine from the motor nerve terminals without affecting the response of the muscle to the chemical transmitter. β-Bungarotoxin consists of two dissimilar polypeptide chains, A chain with 120 amino acid residues and B chain with 60 residues, crosslinked by an interchain disulfide bond and possesses a weak phospholipase A_2 activity. The amino acid sequence of the A chain is homologous with that of the single chain notexin from the venom of Australian tiger snake *Notechis scutatus scutatus*, basic subunit of crotoxin from South American rattlesnake *Crotalus durissus terrificus* and all three subunits of taipoxin from Australian snake Taipan *Oxiuranus scutellatus scutellatus* venom.

All presynaptically acting neurotoxins are basic phospholipase A_2 (PLA_2) *per se* or contain a basic PLA_2 as an indispensable part of their structures. An approach to elucidate the relationship between toxicity and enzymatic activity of PLA_2 can be achieved by chemical modifications of various amino acid residues in the enzyme molecules. Alkylation of His-48 at the active site in snake venom PLA_2 lead to a dramatic and equal loss of enzymatic activity and lethal toxicity, whereas by means of lysine modification a successful dissociation of enzymatic and pharmacological properties has been achieved. Thus modification of the PLA_2 enzyme with pottasium cyanate at pH 8.0 resulted in selective carbamilation of Lys-residues, showing that the carbamylation did not affect the enzymatic activities but the lethality and various pharmacological activities were drastically reduced or abolished. Modification of carboxylate group, on the other hand, causes loss of enzymatic activities but preservation of considerable pharmacological potency. Thus, it is concluded that the toxicity of pure PLA_2 may be due to a direct non-enzymatic effect which does not correlate with level of phospholipid hydrolysis and that the direct effect is prominent in the relative toxic PLA_2 enzyme.

Regarding the structure and function of cobrotoxin, it is elucidated that on re-

duction with β-mercaptoethanol cobrotoxin displays eight SH group and it lost its lethality and antigenic specificity and conformational change occurred concurrently. Reduced cobrotoxin, however, by gentle air oxidation, yields a biologically active product showing essentially the same ORD and CD curves as those of native toxin. It is also indicated that when the invariant Tyr-25 which appears to be "buried" in the molecule was modified in the presence of 5 M gusnifine-HCl, the toxin lost its biological activity. These facts suggest that the integrity of the disulfide bonds and the Tyr-25 in cobrotoxin is essential for its biological functions.

It is also elucidated that by the selective chemical modification, two cation groups of ε-amino group of Lys-47 and guanidino group Arg-33, both of which are common to all known postsynaptic snake neurotoxin conjugate specifically with the acetylcholine receptor on the motor endplate and result the blockade of neuromuscular transmission.

4 SNAKE BITE AND VENOM PROCOAGULANTS

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Snake venom contains several proteases, including endopeptidases, peptidases, proteinases with a limited specificity such as thrombin-like enzyme, prothrombin activator, Factor X and IX activators, kininogenase and arginine ester hydrolase. Most of these proteases, with the exception of peptidases, are mainly found in crotalid and viperid venoms and closely associated with some pathological action, such as hemorrhage, thrombosis, hypotension or edema formation induced by these snake bites. The occurence of hemorrhage is one of the most striking consequences of the snake bite. Bleeding in several organs such as the muscle tissues, brain, lungs and kidneys is often encountered. There are many evidences that such severe hemorrhage is induced by the endopeptidases contained abundantly in crotalid venoms. It is of interest to note that most of the venom endopeptidases inactivate specifically plasma protease inhibitors, such as α_1-antitrypsin and antithrombin III.

On the other hand, it is well known that crotalid and viperid venoms contain several procoagulants which induce activation of the blood coagulation factors, fibrinogen, factor X, factor IX, factor V, protein C and prothrombin, although there is a singificant difference in the procoagulant contents of each venom. These procoagulants modify the blood coagulation system as well as the physiology of hemostasis and result in thrombosis. One of the most well-known venom procoagulant is a factor X-activating enzyme found in the venom of *Vipera russelli*. This procoagulant, in addition to thrombin-like enzyme, such as reptilase, ancrod and batroxobin, have been used as therapeutic agents. The prothrombin activator contained in the venom of *Echis carinatus* is also well-known. The activator catalyzes the prothrombin to α-thrombin. These components have been extensively purified and characterized biochemically. In this symposium, I have described a recent

progress of biochemistry of the venom procoagulants listed in Table.

Table Snake venom components associated with hemorrhage and thrombosis

Components	Venoms	Mol. Wt.	Biological Functions
Platelet aggregation factor	*T. okinavensis* *T. flavoviridis*	400,000	induces platelet aggregation and release reaction
Thrombin-like enzyme	*B. atrox*, *C. adamanteus*	37,000	releases only fibrinopeptide A in the conversion of fibrinogen to fibrin
Prothrombin activator	*E. carinatus* *Notechis scutatus* *Rhabdophis tigrinus*	57,000	catalyzes the conversion of prothrombin to α-thrombin
Factors X, IX and protein C activators	*Vipera russelli*	79,000	catalyses the activation of Factors X, IX and protein C
Kininogenase	*A. halys blomhoffii* *Bitis gabonica*	35,000	releases bradykinin from plasma kininogens
Anticoagulant	*A. acutus*, *T. gramineus*		inhibits the conversion of prothrombin to α-thrombin by Factor Xa
Endopeptidases	Crotalid and Viperid venoms	24,000~ 95,000	inactivates α_1-antitrypsin and antithrombin III
Factor XIII activator	*B. asper*, *B. moojeni*		activates plasma protransglutaminase (Factor XIII)
Hemorrhagic factors	Crotalid and Viperid venoms	24,000~ 95,000	induces lysis of connective tissue and basement membrane
Arginine ester hydrolases	*A. halys blomhoffi*, *C. atrox*, *C. viridis*	35,000	increases capillary permeability
Phospholipase A_2	Elapid, Crotalid and Viperid venoms	14,000	hydrolyzes phospholipids and induces hemolysis

T: *Trimeresurus*, *B*: *Bothrops*, *A*: *Agkistrodon*, *C*: *Crotalus*.

5 MYOGLOBINURIA CAUSING PHOSPHOLIPASES A IN SNAKEBITE

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Myoglobinuria is the most conspicuous symptom in sea snake (*Enhydrina schistosa*) as well as in several Australian Elapidae snake envenomations. The active principle in *E. schistosa* venom producing myoglobinuria has been characterized as a basic phospholipase A. This paper reports the fractionation of 10 Australian snake venoms. Ten Australian snake venoms (*Oxyuranus s. scutellatus*, *Notechis s. scutatus*, *N. ater serventyi*, *N. a. humphreysi*, *Acanthophis antarcticus*, *Austrelaps superba*, *Pseudonaja textilis*, *Pseudechis australis*, *Pseudechis porphyriacus* and *Pseudechis colletti*) were fractionated by column chromatography on CM-Sephadex C-25 and the isolated fractions were tested in mice for ability to produce myoglobinuria after s.c. injection. Only venom fractions from *A. superba*, *P. australis*, *P. porphyriacus* and *P. colletti* produced

this effect. Pure factors were obtained from the latter three venoms and shown to be neutral or basic phospholipases A containing 120–129 amino acid residues (formula weight between 13,400 and 14,200). The enzymes have acute LD_{50} values (s.c. injection) of 4.3–7.7 mg/kg, but the minimum doses to produce myoglobinuria in mice are between 0.5 and 5.0 mg/kg. Phospholipase A (fraction II) from *Pseudechis colletti* guttatus venom caused a dose dependent increase of Creatin Kinase level and myoglobinuria. The amino acid compositions of the four phospholipases A from *P. porphyriacus*, *P. australis* and *P. colletti* show many features in common as compared to the myotoxin from *E. schistosa* venom, mulgotoxin a from *P. australis* and notexin from *Notechis s. scutatus* venom: total number of residues between 119–129, high content of aspartic acid, glycine, alanine, lysine and mostly 14 half-cystine residues suggesting seven disulfide bridges.

Several of the most potent venom toxins are either basic phospholipases A or contain a subunit of phospholipase A. To this group belong presynaptic toxins, myoglobinuria factor, cardiotoxins and toxic phospholipases A. There is no striking dissimilarity among these phospholipases A in amino acid compositions, the sequences around the N-terminal regions (Interface recognition site) and the enzymatic active site (His 48). Thus the specificity for toxic and pharmacological effects must reside somewhere else in molecules.

Group reports

Virological and epidemiological studies on encephalitis in northern part of Thailand

1 VIROLOGICAL AND EPIDEMIOLOGICAL STUDIES ON ENCEPHALITIS IN CHIANG MAI AREA, THAILAND, IN THE YEAR OF 1982 –OUTLINE OF THE STUDY, VIRUS ISOLATION AND ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)–

AKIRA IGARASHI[1], TAKAYUKI OGATA[2], NOBUYA FUJITA[3], TOSHIHIKO FUKUNAGA[4], AKIO MORI[5] AND YOSHIO UZUKA[6]

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Studies were performed during July and August, 1982, in Chiang Mai area, Northern Thailand, in order to obtain recent informations on encephalitis, by isolation of viruses from clinical materials and field-caught mosquitoes, serological examination on hospitalized patients, healthy humans and various vertebrates, and entomological studies on vector mosquitoes of Japanese encephalitis (JE) virus. Virus isolation was performed by inoculation to *Aedes albopictus*, clone C6/36, cells. Eleven strains of dengue viruses (8 type 1, 2 type 2, and 1 type 3 viruses) from 177 peripheral bloods of hospitalized patients. Nine of the isolates were obtained from dengue hemorrhagic fever (DHF) patients, but one of the type 1 virus was obtained from encephalitis, and one of the type 2 virus from aseptic meningitis patient, respectively. While, JE virus was isolated from one of the three postmortem brain materials of encephalitis patients, but isolation from 15,513 mosquitoes of *Culex tritaeniorhynchus*, *Cx. gelidus* and *Cx. fuscocephala* in 125 pools turned out to be negative.

Serological examination on patients' sera showed that hemagglutination inhibition test did not always give clear-cut results because of the cross-reactivity between JE and dengue viruses and did not give high efficiency of serodiagnosis. Positive IgM-ELISA against JE antigen was observed in 27 out of 55 encephalitis cases with totally negative results with 79 DHF cases. Seroepidemiological survey on healthy humans showed that the positive rates of IgM-ELISA against JE antigen

were significantly different among five sampling places, indicating the inhomogeneity of recent infection rate by JE virus throughout the study area.

Many of the Hill Tribe people were found to have suffered from encephalitis. Because of their cultural habitats, which do not possess watered rice fields, the mechanism or route of infection of these people would be worth-while to study in the future.

2 VIROLOGICAL AND EPIDEMIOLOGICAL STUDIES ON ENCEPHALITIS IN CHIANG MAI AREA, THAILAND, IN THE YEAR OF 1982 –ANALYSIS OF THE ADMITTED PATIENTS–

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From July 12 to August 18, 179 hospitalized patients with clinical diagnosis of encephalitis (55 cases), meningitis (8 cases), Dengue hemorrhagic fever (D.H.F.) (77 cases), unknown fever (11 cases) and other diseases (28 cases) were examined in Chiang Mai area, Thailand. Largest number of encephalitis cases were observed in the age group of 10–14 years old, whereas, the patients with D.H.F. were most numerous in the age group of 20–29 years old. These age distributions basically shifted toward older age group, comparing with those in Thailand as a whole. The distribution of encephalitis patients was different from those of D.H.F. patients according to their home address. Patients with D.H.F. appeared to be concentrated in and around Chiang Mai City, whereas, those with encephalitis were observed throughout Chiang Mai Valley, and even in the woodlands. All blood specimens collected from these 179 patients were serologically examined on the antibody titer against JE virus and dengue viruses, and also cultured to isolate arboviruses. Although the details of these results will be reported separately, so many cases of Japanese encephalitis and D.H.F. were confirmed. And JE virus was isolated from one of the three postmortem brain specimens.

From this study, recent epidemic of Japanese encephalitis in Chiang Mai area, Thailand, was confirmed, and the characteristics of this epidemic, i.e., age distribution, geographical distribution, and so on, were revealed.

3 VIROLOGICAL AND EPIDEMIOLOGICAL STUDIES ON ENCEPHALITIS IN CHIANG MAI AREA, THAILAND, IN THE YEAR OF 1982 –SERODIAGNOSIS AND SEROEPIDEMIOLOGY–

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In July-August 1982, a human seroepidemiological study was conducted in Chiang Mai area, Thailand. Paired sera collected from 98 patients hospitalized with encephalitis, dengue hemorrhagic fever and others, and 954 sera from healthy indigenous residents in four study areas (Sarapee, Doi Saked, Mae Taeng and Pasang), near Chaing Mai City, in the Chiang Mai Valley and one study area (Fang), located approximatery 160 km north of Chiang Mai City, in the basin of the River Mae-Khong were tested for hemagglutination inhibition (HI) antibody against Japanese encephalitis (JE) and dengue (DEN) type 1, 2, 3 and 4 viruses.

Antibody to JE virus was comparatively highly prevalent in localities of Mae-Tang and Pasang, rare in Fang. DEN antibody was highly prevalent in all four study areas in Chiang Mai Valley, except in Fang. Compared with data (1969–73) reported by SEATO Institute and other investigators, there is every indication that a prevalence of DEN virus has been increasing in Chiang Mai area since 1970, particularly in the Chiang Mai Valley, rare in the basin of the River Mae-Khong.

4 A SURVEY OF NEUTRALIZING ANTIBODIES TO JAPANESE ENCEPHALITIS AND DENGUE VIRUSES OF HEALTHY PEOPLE IN CHIANG MAI AREA, THAILAND

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A total number of 985 blood specimens collected from healthy people at five districts, four (Pasang, Sarapee, Doi Saket, Mae Taeng) locate in the Chiang Mai

Valley and another district, Fang, locates in another valley, which is 160 km far and separated by mountainous zones from the Chiang Mai Valley. People were devided into nine age-groups; 1–3, 4–6, 7–9, 10–14, 15–19, 20–29, 30–39, 40–49, and over 50 years, and about 20 specimens were collected in each age-group in each district. The neutralization tests were performed by the 50 per cent focus reduction method, employing peroxidase-anti-peroxidase staining technique (Biken J., 21, 137–147).

The results of the tests were analyzed and summarized as follows: (1) The serum specimens from the four districts in the Chiang Mai Valley showed similar patterns of the positive ratios of neutralizing antibodies to Japanese encephalitis (JE) and dengue viruses by the age-group and at the age of 10–14 years the ratios reached 70 per cent or higher, except the ratio to dengue type 2 virus, which appeared lower. (2) The serum specimens in Fang District showed a pattern of the increasing positive ratio of neutralizing antibody to JE virus with increase of age as seen with those in the Chiang Mai Valley, though the positive ratio increased less rapidly with increase of age than those in the Chiang Mai Valley. The positive ratios of neutralizing antibodies to dengue viruses did not show the increase with increase of age, indicating that the prevalences of dengue type 1–4 viruses in Fang District were much lower than those in the Chiang Mai Valley. (3) By the analysis of serum specimens with positive neutralizing antibody to a single type of dengue viruses, it was demonstrated that all four serotypes of dengue viruses had existed in all five districts. (4) At present, the major part of infantile population in the Chiang Mai area, including Fang District, seems to be exposed to dengue viruses first and then infected with JE virus.

5 ANTIBODY SURVEY ON ANIMALS IN CHIANG MAI AREA, THAILAND, 1982

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During a study period from July 19 to August 17, 1982, in Chiang Mai area, serum specimens were collected from various vertebrates in order to measure antibody titers against Japanese encephalitis (JE) and Dengue viruses by the hemagglutination inhibition (HI) test. The purpose is to know the antibody prevalence among these vertebrates which might serve as hosts of JE virus. Some of them may be an effective amplifier of the virus like swine in Japan (Scherer *et al.*, 1959; Konno *et al.*, 1966).

Taking the HI titer of 10: 1 as a positive antibody limit, the antibody positive

rate as measured by JE antigen is high in horses (100%), mules (100%), swine (97%), sheep (93%), dogs (93%), however the rate is low in monkeys (10%), ducks (17%), sparrows (3%), and none in chikens and lizards.

The antibody prevalence rate decreased when HI titers were measured by Dengue-1 antigen to the following values: swine (82%), dogs (80%), sheep (57%), mules (31%), horses (20%), ducks (13%), monkeys (10%), respectively. High antibody positive rate among horses and mules is reasonable, since they are susceptible to JE virus (Clarke and Casals, 1965), and their titers were even higher than that in swine, the most important amplifier. High antibody positive rate and high titer of HI antibodies among sheep and dogs were remarkable.

HI antibodies measured by Dengue might be ascribed to the cross reacting antibody after JE virus infection, since the antibody titer to JE antigen was significantly higher than that to the Dengue antigen. There remains a possibility that their antibodies were made by both virus infections.

6 STUDIES ON BIONOMICS OF *Culex tritaeniorhynchus*, *Culex gelidus* AND *Culex fuscocephala* AND VIRUS ISOLATION IN CHIANG MAI, THAILAND

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Collections of mosquitoes were made by using light traps at four collection sites for the period from July 13 to August 12 around Chiang Mai City, and virus isolation was attempted from the collected mosquitoes using C6/36 clone *Aedes albopictus* cells.

Four thousand nine hundred and thirty five *Culex tritaeniorhynchus*, 7,052 *Cx. gelidus* and 3,526 *Cx. fuscocephala* were sorted from the collected mosquitoes and processed for virus isolation. Two strains of unidentified flavivirus were isolated from *Cx. tritaeniorhynchus* and *Cx. gelidus*, and 59 unidentified filtrable agents which produced various extent of cytopathic changes to C6/36 cells were found. From the present result and findings of Gould *et al.* (1974), it is clear that the infection rate of these mosquitoes with JE virus is lower in Chiang Mai area than in Japan.

In order to elucidate the factors causing the low infection rate, host preference was tested on the source of blood meals in freshly engorged females. The results of this observation indicated that a lot of these mosquitoes fed porcine blood. This result did not agree with Gould and co-workers' data that these mosquitoes prefer buffalo and cattle to pigs or humans, and that the bovine population serves to dampen the spread of JE virus in Chiang Mai area.

Estimations were attempted for the daily survival rate of *Cx. tritaeniorhynchus* females based on the data of parous rates in the field and the assumption that the gonotrophic cycle to be three or four days. Thus, the daily survival rate is estimated as 0.600 or 0.682 respectively. This values of survival rates were approximately

similar to the results of Buei and Ito (1982) and Wada *et al.* (1969) in Japan.

Therefore, the low infection rate of these mosquitoes with JE virus in Chiang Mai area cannot be explained by shorter life span of the mosquitoes. It may be considered that a large number of these mosquitoes emerging in Chiang Mai area could cover their low infection rate with JE virus, resulting in many apparent cases.

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