ISSN 0304-2146

日本熱帯医学会雑誌

Japanese Journal of Tropical Medicine and Hygiene

第15巻 第2号 昭和62年6月15日

内 容

日熱医会誌
Japan. J.T.M.H.

日本熱帯医学会

Japan. J. Trop. Med. Hyg., Vol. 15, No. 2, 1987, pp. 87-96

EPIDEMIOLOGICAL IMPORTANCE OF COP REACTIONS IN AREAS LIGHTLY INFESTED WITH *SCHISTOSOMA JAPONICUM* IN THE PHILIPPINES

HIROSHI TANAKA[1], ALFREDO T. SANTOS, JR.[2], HAJIME MATSUDA[1], KAZUO YASURAOKA[3], BAYANI L. BLAS[2], MINORU NAKAO[4], KUNIOKI ARAKI[5] AND SATOSHI NAKAMURA[1]

Received March 25 1987/Accepted May 15 1987

Abstract: Dwellers in 6 villages in Talibon and Trinidad in Bohol, Philippines with an approximate population of 5,740 were examined for schistosomiasis japonica by stool examination and circumoval precipitin (COP) test during the period from 1981 to 1984. Eggs were positive in 3.5% of 4,117 examinees, whereas COP positives were 9.8% in 4,094 examinees. Since prevalence of egg positives among COP positives increased from 24.4 and 24.0% to 79.1 and 48.1%, in 2 villages after repeated stool examinations, respectively, COP reactions by standardized method seem to indicate the actual infection of individuals. Among 6 villages, the prevalence did not vary much between 1.4% to 6.9% by a single stool examination while there was greater variation between 3.2% and 22.2% by COP test. Two villages, San Vicente and Sto. Tomas, were identified as the area of highest risk. At San Vicente where no snail colony had previously been found; a new snail colony was first identified at Apao Palawan Swamp in July 1984. The distribution of households of infected persons showing strong COP reactions led to the suspicion of presence and the eventual discovery of the snail colonies in the area.

INTRODUCTION

Schistosomiasis japonica is a major health problem in the Philippines, having more than 600,000 of estimated human infections in endemic areas in 1975 (Santos, 1976). Its endemicity on Bohol Island was first proven in 1958 by Blas and Dazo (1968). Schistosomiasis was found in localized areas with an exposed population of 6,000 in 6 villages with a prevalence of medium level at that time. Later, the infestation was reported to be as low as 5.4% in 1980 (Carney *et al.*).

1 Department of Parasitology, Institute of Medical Science, the University of Tokyo, Minato-ku 108, Tokyo
2 Schistosomiasis Control Service, Department of Health, Sta Cruz, Manila 2805, the Philippines
3 Department of Medical Biology, Institute of Basic Medical Sciences, University of Tsukuba, Sakura-mura 305, Ibaraki-ken
4 Department of Parasitology, Asahikawa Medical College, Asahikawa City 078, Hokkaido
5 Division of Parasitology, Department of Microbiology, Institute of Public Health, Minato-ku 108, Tokyo

This study was performed as a part of a collaborative project of the Schistosomiasis Control Service, Department of Health, Philippines and Sasakawa Memorial Health Foundation, Japan for schistosomiasis control on Bohol Island, the Philippines

The present program was designed and commenced in 1981 to control schitosomiasis almost completely at these lightly infested areas by means of selective mass-chemotherapy with praziquantel supplemented with snail control by molluscicide. In this program, all dwellers in 6 infested villages were examined by circumoval precipitin (COP) test and stool examination from 1981 to 1984. Results showed that the prevalence in this area was not so low as had been reported previously. While working for case detection, important roles of COP test were clarified in the epidemiological surveys.

The present study demonstrates the value of COP test in case detection as an important parameter and in finding the snail habitat as the hidden source of infection.

Materials and Methods

Locality studied: The area studied consisted of 6 Barangays (villages) in 2 Municipalities, *i.e.*, San Agustin and San Roque in Talibon and San Vicente, Mabuhay Cabigohan, Sto. Tomas and Kinan-oan in Trinidad which are situated on the northeastern part of Bohol Island. Bohol is

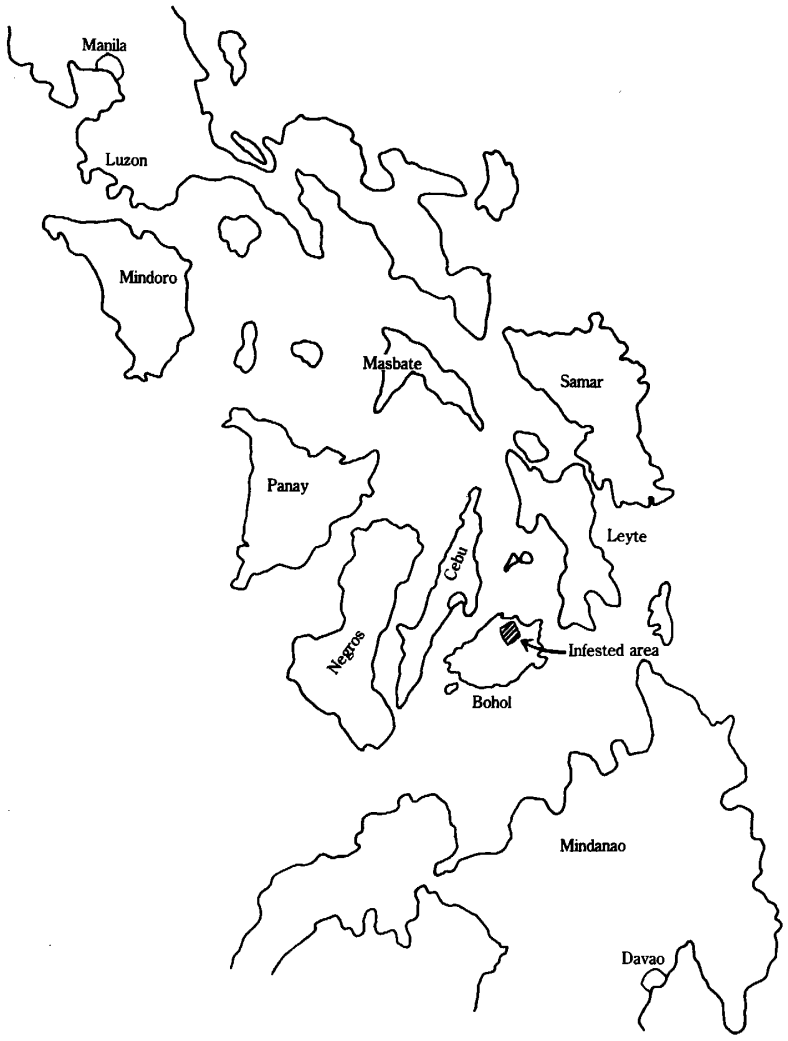


Figure 1 Map of the Philippines showing the infested area on Bohol Island.

located southwest of Leyte and east of Cebu, Philippines (Figure 1).

The total exposed population in the 6 villages is about 6,000. The area is in a tropical climate with much rain the whole year, especially during the rainy season from July to February. Villages are distributed around a cluster of low limestone hills. Rice fields were developed at depressed narrow belts among hills through which streams and rivers flowed. These water pathways originated from a small pond or a boggy area often covered with naturally growing Palawans. The water is supplied from small springs to these wet areas throughout the year possibly connected with underground water in the hills.

Stool Examination: Stools were examined by the quantitative cellophane thick smear method (Kato-Katz). The amount of stool specimens taken quantitatively in a hole of a punched cardboard for examination was 54.5 mg.

COP test: Blood was collected into a heparinized capillary tube by finger prick and closed with sealing clay at one end. Tubes containing blood were centrifuged late in the afternoon on the same day and kept standing in a refrigerator. The following morning plasma was separated by cutting tubes and used for COP test.

COP reactions were performed following the technique by Matsuda *et al.* (1977) by which sensitivity was 97.3% using plasma among egg positive persons, and gave no false positive reaction. Lyophilized eggs were used as antigen and results were read 48 hours after incubation in a wooden box heated with electric bulbs at 37°C in the day time and not heated at night.

Survey Procedures: Every year, a total of about 1,000 people in 1 or 2 villages were examined. Before sample collection, composition of households was surveyed in the target population and each person was given an individual number. In surveys up to 1984 before mass chemotherapy in each village, people were examined for eggs in stool and COP reactions with plasma. For mass chemotherapy, praziquantel at a dose of 20 mg/kg was given 3 times in a day.

Results

1. Detection of Eggs among COP Positives

Attempt was made to prove actual infections among COP positives by repeated stool examinations (Tables 1 and 2). In San Vicente Village, the number of COP positives was 103 in 1984. Among then, 86 were examined for *Schistosoma* eggs in stool by Kato-Katz method, and 21 were found to be positive for eggs during the 1st examination. In the 2nd stool examination,

Table 1 Increase of egg positives among 86 COP positives after repeated stool examinations by Kato-Katz method at San Vicente Village, Trinidad, Bohol, Philippines in 1984

Repetition	No. exam.	No. egg+	Cumulative	
			+ no.	% +*
1	86	21	21	24.4%
2	51	43	64	74.4
3	4	4	68	79.1
Total	86	68		

* Percentage of an initial 86 COP positives.

eggs were found in 43 additional patients out of 51 stool samples submitted out of 65 egg negatives during the 1st examination. In the 3rd examination, 4 cases out of 4 samples submitted were further found to be egg positive. Of the COP positive group 24.4% were positive for eggs during the 1st examination and this rose to 79.1% after a 3rd repeat examination (Table 1).

In the same way, 104 COP positives were examined repeatedly for *Schistosoma* eggs by MIFC method at San Roque in 1985, where infestation was lower than in San Vicente Village. In this positive group, 11 cases who had been treated with praziquantel before 1985 were excluded. At each fecal examination, those who were not detected positive for eggs were requested for further repeated examinations. By the 5th repeat stool examination, 24.0% of egg positives among COP positives at the 1st stool examination increased to 48.1% (Table 2).

Table 2 Increase of egg positives among 104 COP positives after repeated stool examinations by MIFC method at San Roque Village, Talibon, Bohol, Philippines in 1985

Repetition	No. exam.	No. egg+	Cumulative	
			+ no.	% +*
1	104	25	25	24.0%
2	49	10	35	33.7
3	29	7	42	40.4
4	15	6	48	46.2
5	4	2	50	48.1
Total	104	50		

* Percentage of an initial 104 COP positives.

Since the number of egg positives increased with the repetition of stool examinations and there were likely non egg-passers among infected people, COP reactions by this standardized method were considered to indicate the level of infection which was closer to the actual level than indicated by a single stool examination.

2. Difference of Infestations among 6 Villages

In the period from 1981 to 1984, most dwellers in 6 infested villages were examined by both stool examination and COP test (Table 3). Since the COP test was not conducted at San Roque in 1981, date in 1985 is presented in Table 3. The population within project area based on the household survey was 5,735 out of which 4,117 at a coverage of 71.8% were examined for eggs and 4,094 or 71.4% were examined by COP test.

In Table 3, results of repeated stool examinations among COP positives at San Vicente and Kinan-oan are presented. If results of the repeated stool examinations are disregarded, percent positives by a single stool examination ranged from 1.4% at Kinan-oan to 6.9% at San Vicente and the difference among villages was not so large. The highest prevalence was noticed in San Vicente followed by San Roque and Sto. Tomas.

The results of COP test, however, showed much higher prevalence and a clear difference among villages. The highest prevalence was observed in San Vicente followed by Sto. Tomas

Table 3 Survey results of schistosomiasis japonica by stool examination and COP test in 6 villages before mass-chemotherapy on Bohol, Philippines during the period from 1981 to 1984

Locality	Year	Population	Stool examination			COP test		
			No. exam.	+ no.	% +	No. exam.	+ no.	% +
TALIBON								
San Agustin	1983	1,983	1,137	18	1.6	1,000	32	3.2
San Roque	1981	1,314	1,124	57	5.1	1,182*	95	8.0
TRINIDAD								
San Vicente	1984	542	461	32	6.9	464	103	22.2
			(465**	72	15.5)			
M. Cabigohan	1982	668	448	12	2.7	517	52	10.1
Sto. Tomas	1982	558	430	19	4.4	389	83	21.3
Kinan-oan	1984	670	495	7	1.4	542	37	6.8
			(513**	32	6.2)			
Total		5,735	4,095	145	3.5	4,094	402	9.8
			(4,117***	210	5.1)			

* Performed in 1985.

** Repeated stool examination after COP test.

*** Results including repeated stool examinations.

and M. Cabigohan, where more snail colonies might exist and wider water areas may be contaminated with cercariae.

Results of COP reactions were also valuable in indicating the limited area of highest risk among infested areas.

3. Detection of the Snail Colony

In San Vicente Village, there were no snail colonies hitherto found, although the prevalence of schistosomiasis was revealed to be 22.2% by COP reactions in 1984.

Considering this high prevalence, presence of snail colonies in this village was strongly suspected. Thus, attempts were made to determine the presence of snail colonies based on the epidemiological survey data.

On a sketch map of this village showing distribution of households, egg positives were marked (Figure 2). Positives were found mostly at the southern 1/4 region of this village. On the other hand, COP positives scattered more evenly and widely over the village with lesser distribution at northeast region and dense accumulation at west central and southern regions (Figure 3).

When positives with strong COP reactions (type 3 of Yokogawa *et al.*, 1967 or segmented precipitation of Yogore *et al.* 1968) are taken into consideration (Figure 4), positives are concentrated at the southern region over an area from the fish pond to the upper portion of rice fields which extend from Apao Swamp to San Vicente Creek.

Surveys were made to search for swamps at that area and Apao Swamp with naturally growing Palawans was found. And colonies of intermediate snail host, *Oncomelania quadrasi,*

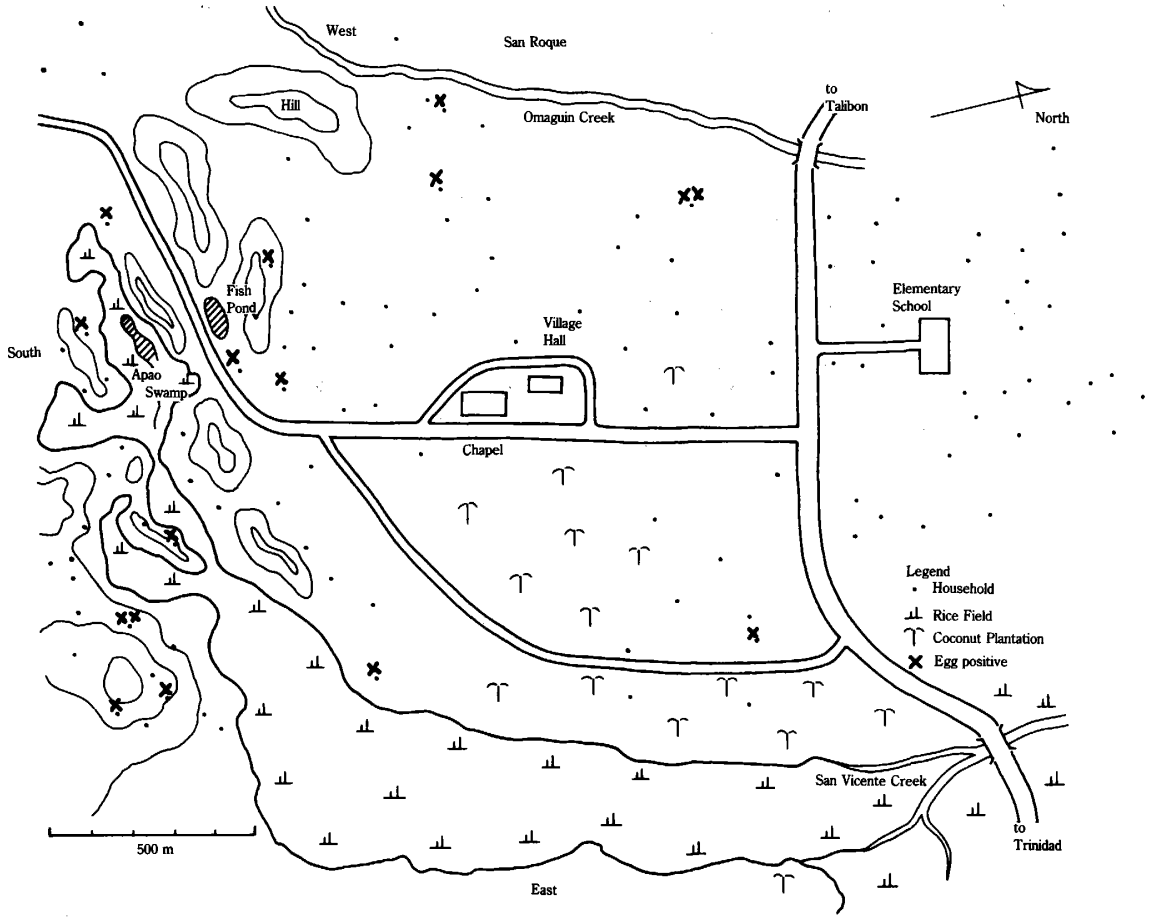


Figure 2 Distribution of egg positives at San Vicente Village in July 1984.

were readily discovered there in July 1984 in this village. However, no more snail habitats were found at that time either at upper portions of rice fields or around fish ponds and connecting canals.

Discussions

COP reactions have been widely used for case detection of infections with *Schistosoma mansoni* and *S. japonicum*. There were a few modified procedures of COP technique for *S. japonicum* (Yokogawa *et al.*, 1967, Yogore *et al.*, 1968, 1979 and Matsuda *et al.*, 1977). The highly sensitive method by Yokogawa *et al.* (1967) was effectively used as the screening diagnostic technique for case finding in an area with a very low prevalence like in Japan.

However, in a highly prevalent area, the sensitive method picks up too many suspected cases among examinees, including a high ratio of false positives. Consequently, nealy all the population should be examined again by another method, more specifically by stool examination, and consequently the COP test looses its value as the 1st screening test.

In schistosomiasis prevalent area, sensitivity of COP reactions should be properly standardized (Yogore *et al.*, 1968, Matsuda *et al.*, 1977) so that false positives are avoided, even if a few may be missed from detection. By the technique of COP test with plasma used in this study,

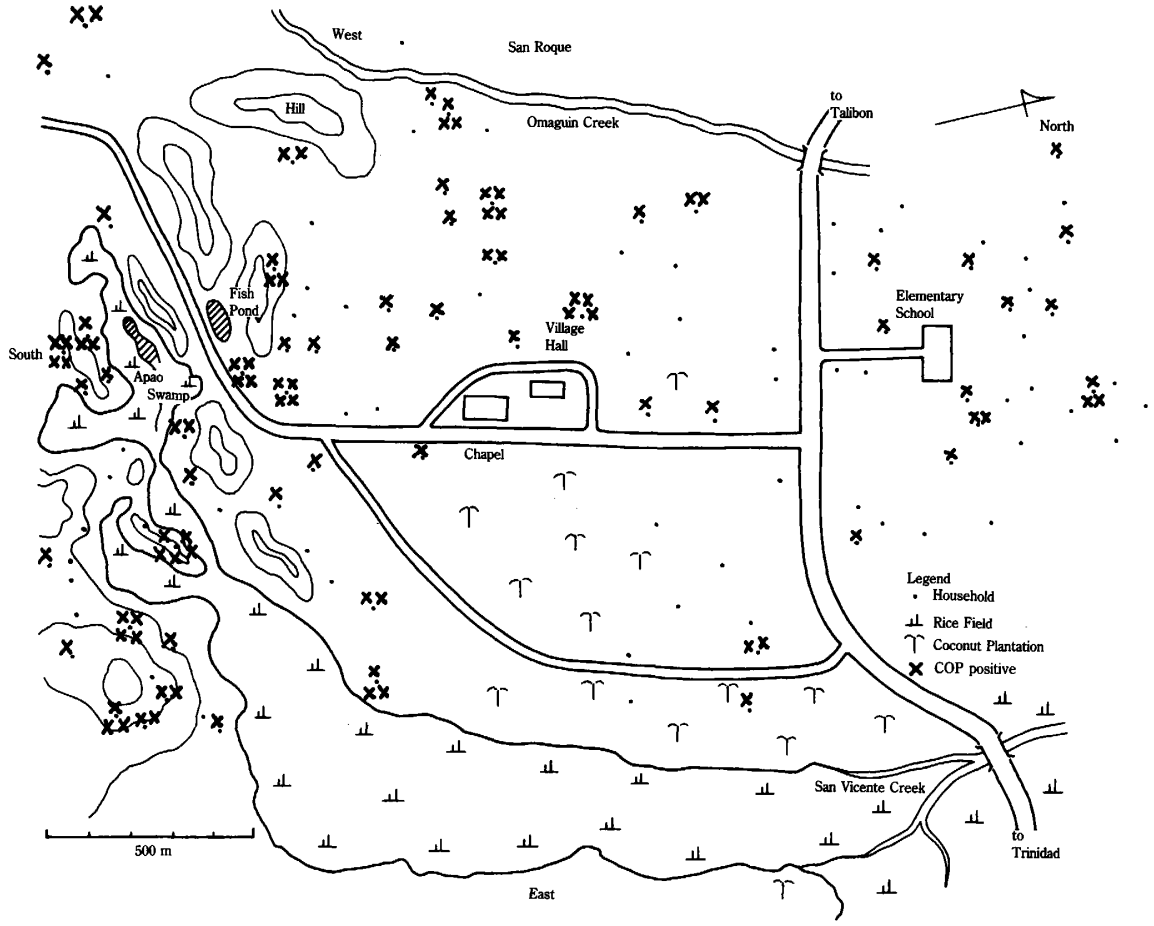


Figure 3 Distribution of all COP positives at San Vicente Village in July 1984.

97.3% of egg positives showed positive COP reactions and there was no false positive (Matsuda *et al.*, 1977).

The COP test is useful in 3 instances at least. Firstly, in epidemiological surveys, the reaction may indicate the actual infection of individual persons, irrespective of evacuation of eggs in stool. In the 2 villages studied which had low infestation, repeated stool examination showed egg positivity rate of 79.1 and 48.1% respectively among COP positives. Yogore *et al.* (1981) compared results by ELISA, COP and stool examination (MFCT), and found additional egg positives after repeated stool examinations only among serologically positive individuals. And they concluded that repeated stool examinations are necessary as the results by stool examination underestimate the true infection.

Since some infected people remain negative for eggs due to light infections, very chronic infections, early stage of infections or shortly after treatment with paraziquantel, it seems that COP positives detected by this technique in this area are persons highly suspected with *Schistosoma* infection and more egg positives will be found by repeated stool examinations or by biopsy method.

The results by COP provide a good indication to a medical doctor in deciding treatment of suspected schistosomiasis even if detection of eggs was negative.

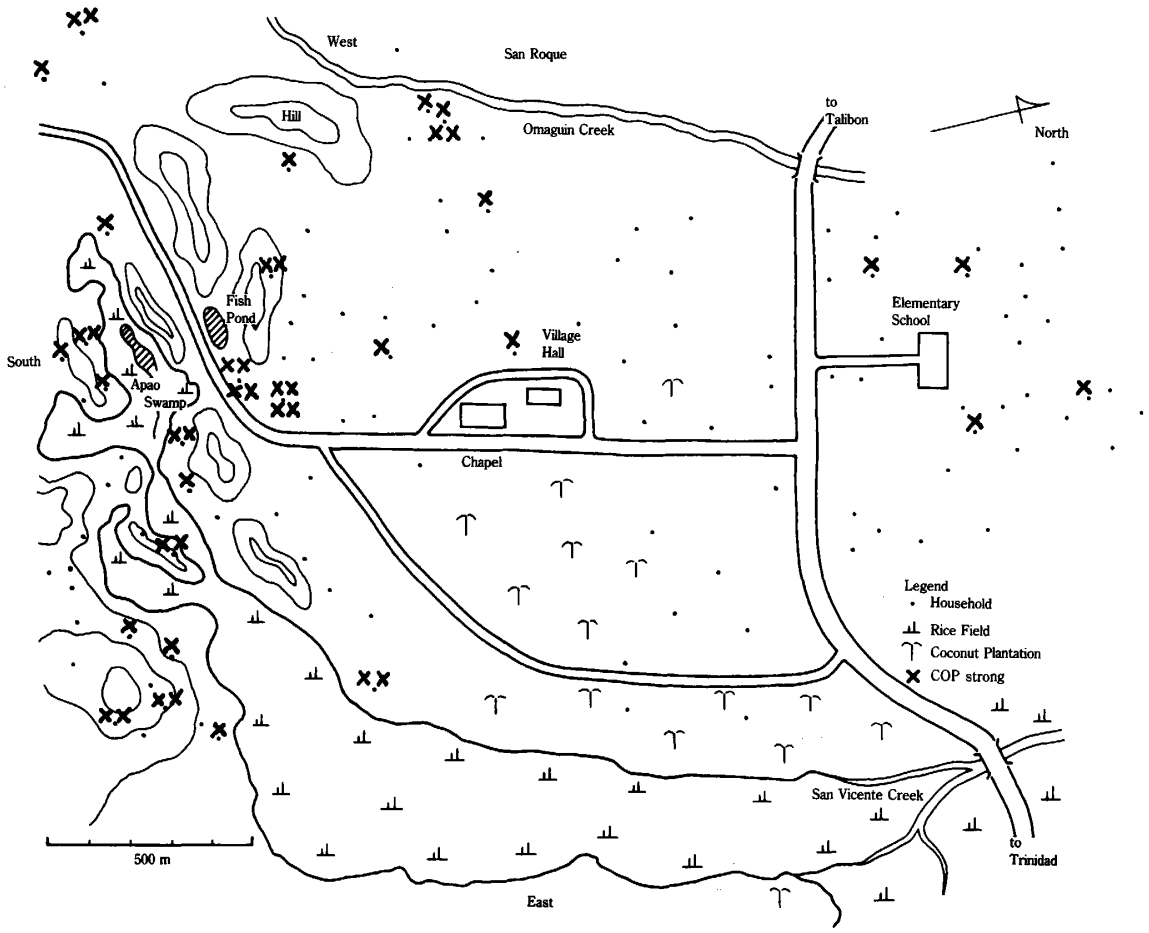


Figure 4 Distribution of strong reactions of COP (Type 3 of Yokogawa *et al.*) at San Vicente Village in July 1984.

The second importance of COP test is to show the infection rate closer to the true rate of target populations which is 3.5% by stool examination and increased to 9.8% by COP test in 6 villages. The small difference in prevalence among villages by stool examination was magnified, and villages with high prevalence were clearly pointed out by COP test. From the results, 2 villages, San Vicente and Sto. Tomas, were identified as the areas of highest risk, and consequently the target area of this control program.

The 3rd application of COP reaction in the epidemiological survey was the discovery of new snail colonies. For this, distribution of strong reactions of COP test was useful. In San Vicente, no snail colonies were found until 1984, even though schistosomiasis among people had been found as early as 1958 by Blas and Dazo (1968). From the distribution of strong COP reactions, suspected foci of infection were delineated in a map, and finally, the snail colony was found at Apao Palawan Swamp in July 1984. This discovery led later to that of 3 more snail colonies around the fish pond and further western region in this village.

Acknowledgments

The present authors wish to show their gratitude to all Philippine members assigned to the

National Schistosomiasis Control and Research Team at Trinidad, Bohol, especially the present team leader, Dr. Victoriano Guiritan, and previous leaders, Mr. Rogelio Pangilinan, Miss Crispina Hinlayagan and Miss Rosalina B. Pacheco.

They would like to dedicate this publication to the late Dr. Julian S. Neseñas who has devoted his whole life to the schistosomiasis control of the Philippines as a staff in the Schistosomiasis Research and Training Center, Palo, Leyte and as the deputy executive director in the Schistosomiasis Contorol and Research Service, Ministry of Health, Manila, and who contributed greatly to this joint program in working on Bohol every year and passed away of heart attack on August 6, 1985.

References

1) Blas, B. L. and Dazo, B. C. (1968): Schistosomiasis survey and preliminary control work in the province of Bohol. J. of Philippine Med. Assoc., 44, 80–87
2) Carney, W. P., Banzon, T., de Veyra, V. and Cross, J. H. (1980): Intestinal parasites of man in northern Bohol, Philippines, with emphasis on schistosomiasis. Southeast Asian J. Trop. Med. Pub. Hlth., 11, 473–479
3) Matsuda, H., Noseñas, J. S., Tanaka, H., Santos, A. T. Jr. and Trinidad-Perez, D. (1977): Comparative studies on reading criteria of circumoval precipitin reaction of *Schistosoma japonicum* for field survey in highly endemic area. Japan. J. Exp. Med., 47, 369–375
4) Santos, A. T. Jr. (1976): Prevalence and distribution of schistosomiasis in the Philippines: A review. Southeast Asian J. Trop. Med. Pub. Hlth., 7, 133–136
5) Yogore, M. G. Jr., Lewert, R. M. and Silan, R. B. (1968): The circumoval precipitin (COP) test in schistosomiasis japonica. Amer. J. Trop. Med. Hyg., 17, 65–71
6) Yogore, M. G. Jr., Lewert, R. M. and Blas, B. L. (1979): Schistosomiasis japonica in Barrio San Antonio, Basey, Samar in the Philippines: III. The plasma circumoval precipitin test-procedure and use in epidemiologic studies. Southeast Asian J. Trop. Med. Pub. Hlth., 10, 23–31
7) Yogore, M. G. Jr., Lewert, R. M. and Blas, B. L. (1981): Schistosomiasis japonica in Barrio San Antonio, Basey, Samar in the Philippines. V. The enzyme-linked immunosorbent assay (ELISA) compared with quantitative stool examination and the circumoval precipitin (COP) test. Am. J. Trop. Med. Hyg., 30, 1252–1262
8) Yokogawa, M., Sano. M. and Araki, K. (1967): Immunosero-diagnosis of schistosomiasis japonica. 3. Circumoval precipitation test. Japan. J. Parasitol, 16, 77–84 (in Japanese with English Summary)

フィリピンの日本住血吸虫症低流行地における COP 反応の疫学的意義

田中　寛[1]・ALFREDO T. SANTOS, JR.[2]・松田　肇[1]
安羅岡一男[3]・BAYANI L. BLAS[2]・中尾　稔[4]
荒木　国興[5]・中村　哲[1]

フィリピン，ボホール島のタリボンとトリニダッド町内の6村落の約5,740人を対象に，1981年より1984年までに日本住血吸虫の虫卵検査と COP 反応を行った。被験者4,094の内，虫卵陽性率は3.5%であったが，COP では9.8%であった。2村落における COP 陽性者の反復した検便の結果，COP 陽性者中の虫卵陽性率は当初の24.4および24.0%から79.1および48.1%へと上昇した。この事から，よく標準化した手法で COP 反応を行えば，住血吸虫の真の寄生率が得られる事が示唆された。6村落間の虫卵陽性率は1.4%から6.9%と差は小さく，COP では3.2%から22.2%と大きな差を示し，サンビセンテとサントトーマスの2村落が最大の危険地域である事が明瞭に示された。サンビセンテでは1984年以前には，中間宿主貝の生息地は発見されていなかったが，COP 反応強陽性者のいる家族の村落内の分布調査から，パラワン芋の茂る，アパオ湿地と呼ばれている場所で多数の貝を1984年7月に初めて発見できた。

1 東京大学医科学研究所　2 フィリピン保健省住血吸虫対策局
3 筑波大学基礎医学系　4 旭川医科大学　5 国立公衆衛生院

Japan. J. Trop. Med. Hyg., Vol. 15, No. 2, 1987, pp. 97-104

THE FATE OF *LEISHMANIA BRAZILIENSIS, L. DONOVANI* AND *TRYPANOSOMA CRUZI* IN DIFFUSION CHAMBERS IMPLANTED INTO HAMSTERS AND MICE —— A PRELIMINARY STUDY ——

YOSHIHISA HASHIGUCHI[1], MASATO FURUYA[2] AND YOSHISUKE OKAMURA[1]

Received January 22 1987/Accepted March 30 1987

Abstract: *Leishmania braziliensis* and *L. donovani* were investigated for the transformation and survival in intraperitoneal (IP), subcutaneous (SC) and intrascrotal (IS) diffusion chambers implanted into hamsters and mice. For a comparison, *Trypanosoma cruzi* was also examined by using the same procedure. The 2 *Leishmania* species revealed an unexpectedly short survival time, and no transformation was observed in the parasites in chambers implanted into hamsters or mice. IS chambers seemed to provide a better condition for *L. donovani, L. braziliensis* and *T. cruzi,* as compared with IP and SC chambers in hamsters. In the study, no IS chambers were examined in mice because of too small size of the scrotum to insert the diffusion chamber. *T. cruzi* showed a considerably longer period of survival than *L. donovani* or *L. braziliensis* in mice, but not in hamsters. The trypanosome, *T. cruzi,* transformed from epimastigote to trypomastigote and amastigote in IP and SC chambers in mice. These results seemed to suggest that the factors responsible for the transformation and survival of the organisms might be greatly different between the 2 genera, *Leishmania* and *Trypanosoma,* and also between the 2 host animals, hamsters and mice.

INTRODUCTION

The parasite of the genus *Leishmania* is an obligatory intracellular organism as amastigote in vertebrate hosts and extracellular one as promastigote in invertebrate hosts. Using such a protozoan, it seems to be interesting to make analysis *in vivo* on the interrelation between the parasite in chambers and the host animals, by implanting diffusion chambers which separate the parasite from a direct physical contact with various host cells by microporous filters. Providing that the organisms survive, and preferably transform and differentiate in the chambers implanted, they provide a useful model for investigating both biology of the parasite and development of immunity in the host. Diffusion chambers allow exchange of various diffusible substances between the parasites in chambers and their hosts. Moreover, if desired, the chamber may be easily retrieved for examination of the implanted organisms. On the basis of these advantages, diffusion chambers have been used to culture *in vivo* several species of parasites, and to immunize host animals against protozoans and helminths.

In *Trypanosoma cruzi,* morphogenesis of the parasite and development of the host immunity

1 Department of Parasitology, Kochi Medical School, Nankoku City, Kochi 781-51, Japan
2 Institute for Laboratory Animals, Kochi Medical School, Nankoku City, Kochi 781-51, Japan

have been studied by using diffusion chambers implanted intraperitoneally into mice (Logan and Hanson, 1974). Moreover, in subcutaneous diffusion chambers, *T. brucei, T. rhodesiense* and *T. gambiense* multiplied rapidly, persisted for as long as 5 weeks and expressed antigenic variation (Ballon-Landa *et al.*, 1985).

In contrast to the genus *Trypanosoma,* no such a study has been attempted in the genus *Leishmania.* In the present study, therefore, a preliminary trial was undertaken to determine whether diffusion chamber is suitable for obtaining information on the interrelation between the implanted parasites and the hosts. As the first step, the transformation and survival of *L. braziliensis* and *L. donovani* were examined in intraperitoneal, subcutaneous and intrascrotal chambers. In the experiment, for a comparison, *T. cruzi* was also examined by using the same procedure.

Materials and Methods

Host animals

Adult male BALB/c mice and Syrian hamsters, *Mesocricetus auratus,* weighing around 30 g and 130 g, respectively, were used. They were fed with a commercially prepared diet and water was provided *ad libitum.*

Parasites and the in vitro cultivation

The organisms studied were 3 species of protozoans, *L. braziliensis, L. donovani* and *T. cruzi.* The 2 species of *Leishmania* were kindly supplied by the Department of Parasitology, Keio University School of Medicine, Tokyo, Japan, and *T. cruzi* was obtained from a patient with Chagas' disease in Guayaquil City, Ecuador. The parasites were maintained at 26°C in a serial *in vitro* passage, as promastigote in *Leishmania* and epimastigote in *Trypanosoma,* in culture medium (Aljeboori, 1979); a part of *in vitro* culture was performed by using Pan's medium (Pan, 1984). No difference was recognized on the transformation and survival between the parasites derived from the 2 culture media used. All the materials used for inoculation of diffusion chambers were from stationary phase of the culture.

Diffusion chambers and the implantation or removal

Diffusion chambers were made of a plexglass ring (U-100, diameter: 10 mm, thickness: 2 mm) and millipore filters (pore size: 0.22 μm). The chambers were constracted by cementing filters to either side of a ring with MF cement (Millipore Filter Co.). Diffusion chambers were sterilized with U. V. for overnight in the experiment. These chambers were aseptically filled with 0.1 m*l* (3×10^5 to 1×10^6 cells) of the parasite suspension. After loading, the access hole of each chamber was sealed with a plastic plug and then covered with a drop of MF cement and allowed to dry before implantation.

For implantation of a diffusion chamber, the hair of the abdominal and scrotal regions of the animals was clipped with an electric clipper, the animals anaesthetized with Nembutal, and the clipped regions were washed with 70% ethanol. An incision about 1.5 cm long was made and then diffusion chamber containing parasites was placed intraperitoneally, subcutaneously and intrascrotally. The incision was closed with silk sutures.

At the removal and examination of these chambers, the animals were anaesthetized and surgically opened, and the chambers were removed and examined for the parasites at intervals.

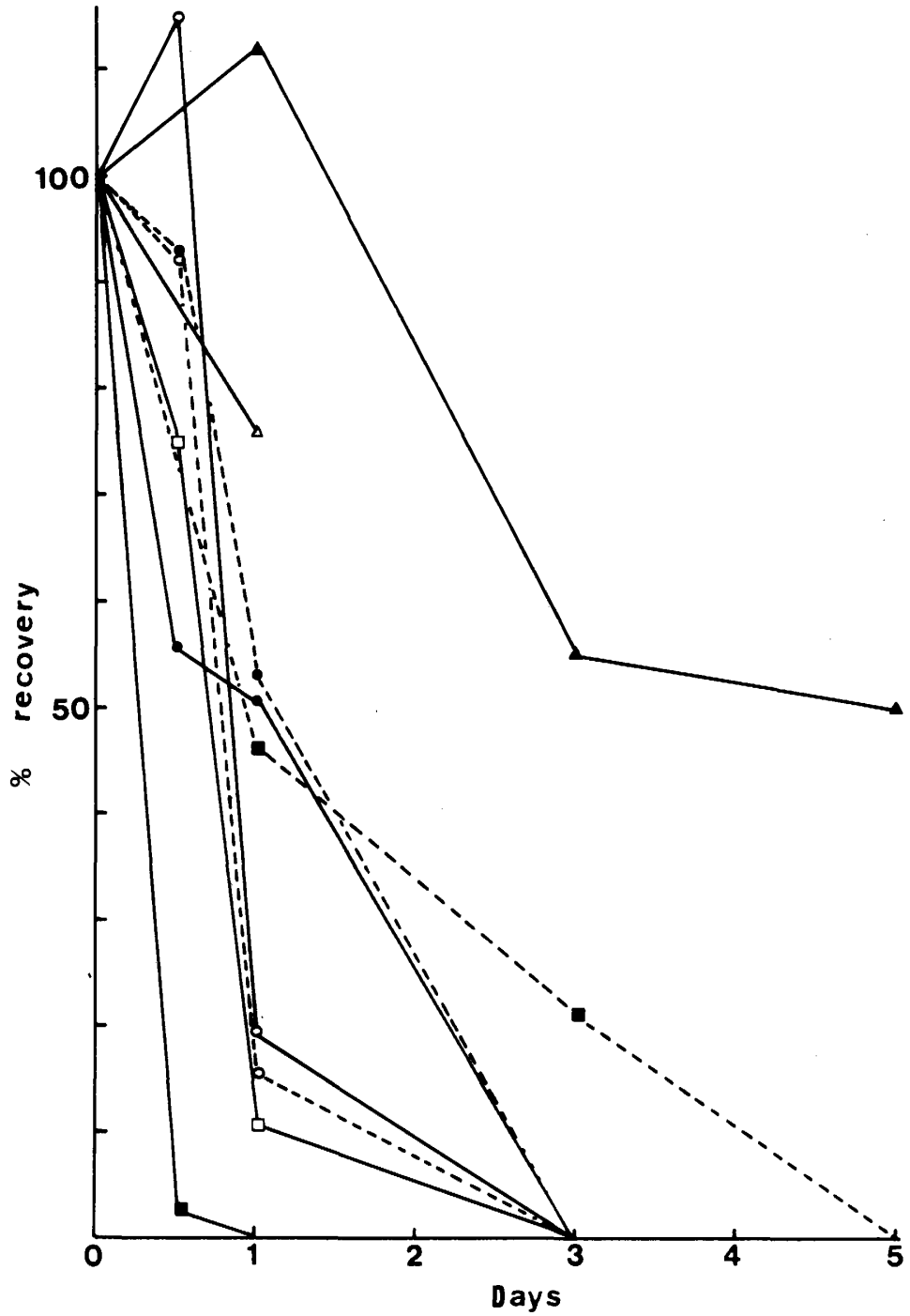
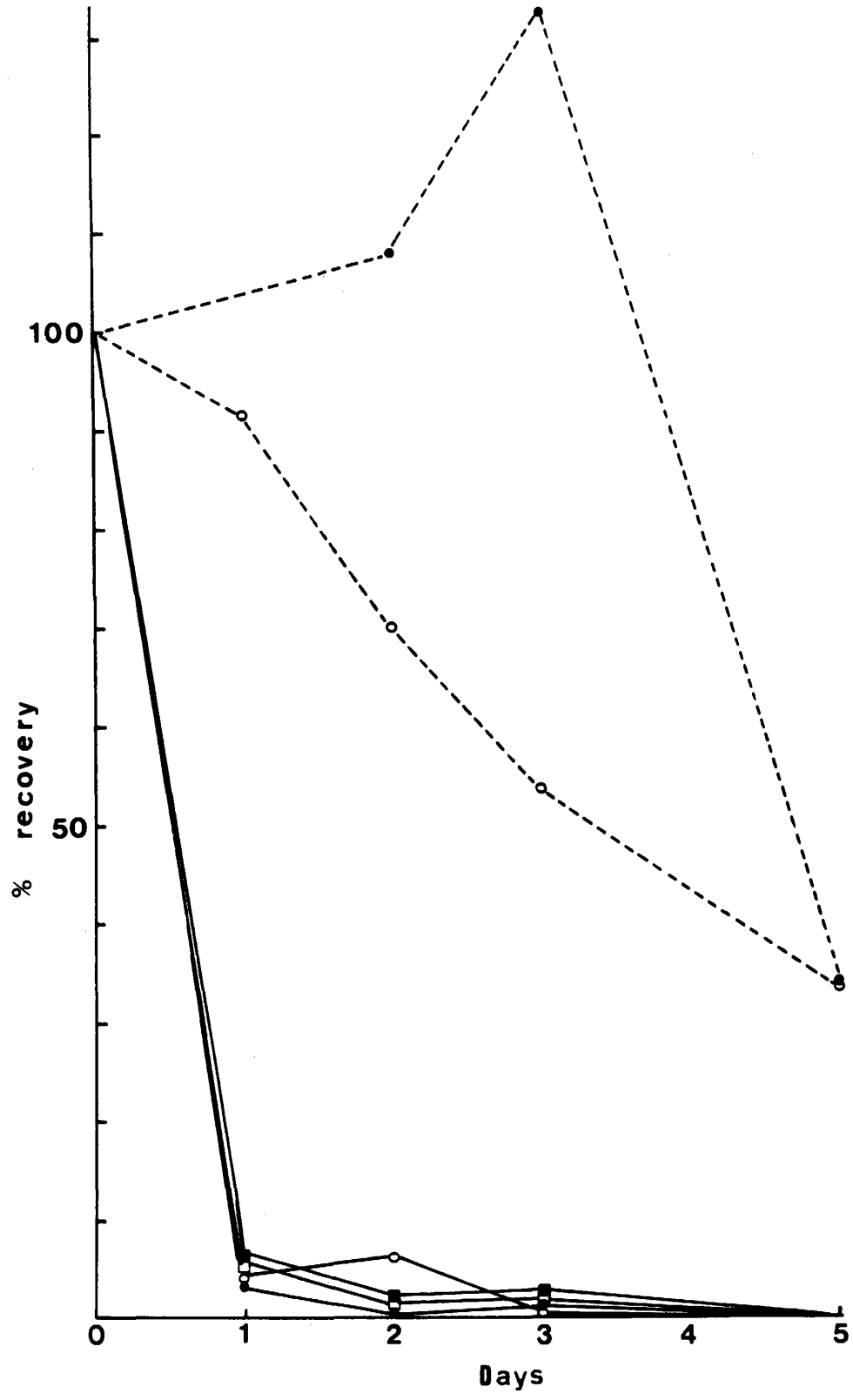


Figure 1 The surviral rate, expressed as % recovery (each point recovery no./initial no. inoculated $\times 100$), of *L. braziliensis*, *L. donovani* and *T. cruzi* in intraperitoneal (IP), subcutaneous (SC) and intrascrotal (IS) chambers in hamsters. Each point shows mean % of 2 to 4 chambers in which 0.1 m*l*/chamber of promatigotes or epimatigotes (3×10^6 to 1×10^7/m*l*) was inoculated. ○——○, *L. braziliensis* in IP chambers; ●——●, *L. braziliensis* in SC; △——△, *L. braziliensis* in IS; □——□, *L. donovani* in IP; ■——■, *L. donovani* in SC; ▲——▲, *L. donovani* in IS; ○----○, *T. cruzi* in IP; ●----●, *T. cruzi* in SC; ■----■, *T. cruzi* in IS.


100
50
% recovery
0
1
2
3
4
5
Days

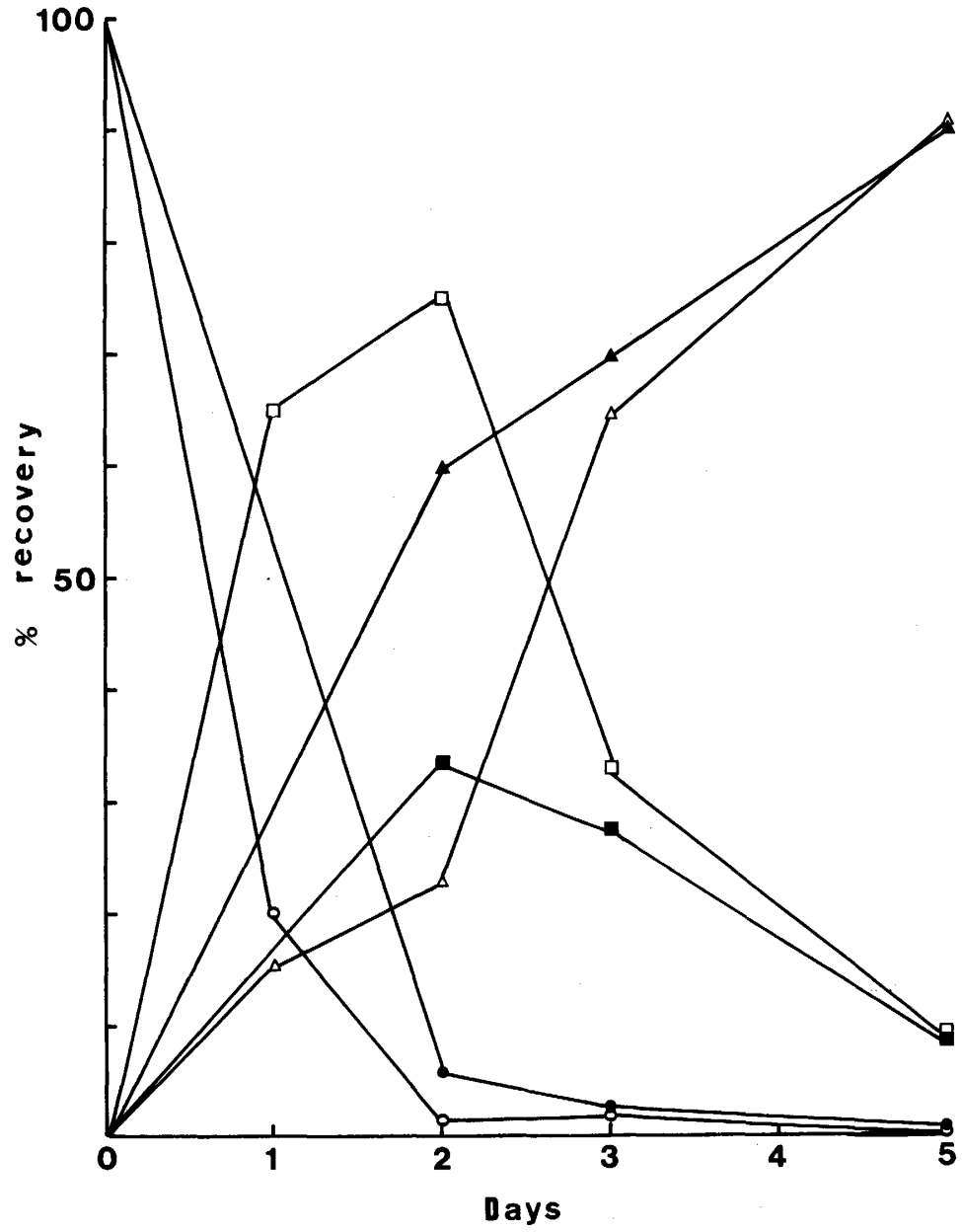


Figure 3 Morphogenetic process of *T. cruzi* in intraperitoneal (IP) and subcutaneous (SC) chambers in mice. Each point indicates the rate, expressed as % recovery, of each stage calculated by counting around 100 protozoans in stained specimens. ○——○, epimastigotes in IP; □——□, trypomastigotes in IP; △——△, amastigotes in IP; ●——●, epimastigotes in SC; ■——■, trypomastigotes in SC; ▲——▲, amastigotes in SC.

Figure 2 The survival rate, expressed as % recovery, of *L. braziliensis*, *L. donovani* and *T. cruzi* in intraperitoneal (IP) and subcutaneous (SC) chambers in mice. Each point shows mean % of 2 to 4 chambers in which 0.1 ml/chamber of promastigotes or epimastigotes (3×10^6 to 1×10^7/ml) was inoculated. ○——○, *L. braziliensis* in IP chambers; ●——●, *L. braziliensis* in SC; □——□, *L. donovani* in IP; ■——■, *L. donovani* in SC; ○----○, *T. cruzi* in IP; ●----●, *T. cruzi* in SC.

A sample of the chamber contents was recovered by insertion of a 27 guage of syringe, and the number of motile parasites was determined by using haemocytometer. An additional sample of the chamber contents was recovered, smeared on a clear microscopic slide, dried, fixed in methanol, and stained in Giemsa's or Wright's staining solution. The morphological stages present in the chambers were studied from these stained specimens with the aid of a compound microscope (1,000×). In some cases, a chamber was examined several times at intervals, by recovering a small quantity of chamber fluid, and then reimplanted into the same host animal.

Results

In hamsters, *L. braziliensis, L. donovani* and *T. cruzi* showed an unexpectedly short survival in intraperitoneal (IP) and subcutaneous (SC) chambers (Figure 1). No marked site preference of the organisms was observed between IP and SC chambers. All the 3 species of parasites, however, tended to demonstrate a longer time of survival in intrascrotal (IS) chambers than in IP and SC ones. In the host animal, no transfomation of the 2 genera, *Leishmania* and *Trypanosoma,* was observed in IP, SC and IS chambers, and the majority of the parasites revealed a small, round and dwarf form with an extremely short flagellum.

In mice, almost all of the leishmanial parasites, *L. braziliensis* and *L. donovani,* have died in IP and SC chambers within 1 day after implantation (Figure 2). *T. cruzi,* on the other hand, survived well in both IP and SC chambers. The organisms in SC chambers multiplied during 2 and 3 days after implantation, but thereafter they gradually decreased and reached 34% of the initial numbers, on day 5. In *L. braziliensis* and *L. donovani,* no transformation of the parasites was observed, while *T. cruzi* transformed into trypomastigote and amastigote in IP and SC chambers, as shown in Figure 3. In IP chambers, transformation of *T. cruzi* from epimastigote to trypomastigote occurred at 65% on the day 1 when 15% of the parasites were recovered as amastigote form. On day 5, around 90% of *T. cruzi* was recognized as amastigote in both IP and SC chambers, but a small rate of the parasites still remained epimastigote or trypomastigote. In the present study no IS chamber was examined in mice, because of too small size of the scrotum to insert the diffusion chamber.

Discussion

The current study was designed to investigate the transformation and survival of *L. braziliensis* and *L. donovani,* together with *T. cruzi,* in IP, SC and IS chambers in hamsters and mice. In the 2 species of the genus *Leishmania,* however, an unexpectedly short survival of the parasites was observed especially in IP and SC chambers in the both host animals, though the parasites in IS chambers revealed a relatively good result. The parasites, furthermore, could not perform transformation and most of them were aberrant forms of the promastigote with an extremely short flagellum. The results obtained indicated that the present condition of chambers implanted was not suitable for *L. braziliensis* and *L. donovani.* But, it was noticeable that the leishmanial parasites tended to survive for a longer time in IS chambers than in IP and SC ones in hamsters. The precise reasons for this better survival were not clear, but it might be due to lower temperature of the scrotum as compared with other body sites, and also due to some immunological and physiological conditions of the organ. It has been well known that the intrascrotal temperature in mammals is considerably below the general body temperature

(Baker, 1986). The intrascrotal region of hamster, therefore, might be a site of choice for the *Leishmania* species in such a study.

T. cruzi, on the other hand, survived well performing morphogenesis in IP and SC chambers in mice, but not in hamsters. As to trasformation of trypanosomes, temperature has been believed as an important factor by several workers. In *T. cruzi,* most of the parasites could be stimulated to transform into trypomastigote, by raising the temperature to 37°C and lowering the pH in axenic culture or tissue culture (Pan, 1971; Trejos *et al.*, 1963). However, there are other facts that *T. cruzi* did not transform in the diffusion chambers either maintained in *in vitro* cell culture at 37°C or in several types of *in vitro* culture media at 26°C or 37°C (Logan and Hanson, 1974). Thus, the influence of temperature might be relatively minor in the transformation of trypanosomes, as compared with other factors (Logan and Hanson, 1974). The morphogenetic process of *T. cruzi* was established when the parasite was maintained in IP chambers in mice (Logan and Hanson, 1974), as same as the present study. Therefore, the factors necessary for the transformation of these protozoans should be further studied in future by using *in vitro* and *in vivo* systems.

From the results obtained, it was concluded that the leishmanial parasites, obligatory intracellular organisms in mammalian hosts, could not survive for a long time and could not perform transformation in diffusion chambers implanted into hamsters or mice. The results also suggested that the factors responsible for the transformation and survival of the organisms might be greatly different between *Leishmania* and *Trypanosoma,* and also between the 2 host animals, hamsters and mice.

Acknowledgements

We thank the late Professor K. Asami, Department of Parasitology, Keio University School of Medicine, Tokyo, Japan for kind supply of *L. braziliensis* and *L. donovani.* Thanks are also due to Drs. N. Suzuki and H. Osaki of Kochi Medical School, Kochi, Japan for their encouragement throughout the study.

References

1) Aljeboori, T. I. (1979): A simple diphasic medium lacking whole blood for culturing *Leishmania* spp, Trans. Roy. Soc. Trop. Med. Hyg., 73, 117
2) Baker, J. R. (1986): Intrascrotal development of trypanosomes, Parasit. Today, 2, 249
3) Ballon-Landa, G., Douglas, H., Colmerauer, M. M., Goddard, D. and Davis, C. E. (1985): Growth and antigenic variation of *Trypanosoma brucei, T. rhodesiense* and *T. gambiense* in subcutaneous millipore chambers, Trans. Roy. Soc. Trop. Med. Hyg., 79, 24–28
4) Logan, L. L. and Hanson, W. (1974): *Trypanosoma cruzi*: Morphogenesis in diffusion chambers in the mouse peritoneal cavity and attempted stimulation of host immunity, Exp. Parasitol., 36, 439–454
5) Pan, A. A. (1984): *Leishmania mexicana*: Serial cultivation of intracellular stages in a cell-free medium, Exp. Parasitol., 58, 72–80
6) Pan, C. T. (1971): Cultivation and morphogenesis of *Trypanosoma cruzi* in improved liquid media, J. Protozool., 18, 556–560
7) Trejos, A., Godoy, G. A., Greenblatt, C. and Cedillos, R. (1963): Effect of temperature on morphologic variation of *Schizotrypanum cruzi* in tissue culture, Exp. Parasitol., 13, 211–218

Diffusion chamber 内 *Leishmania braziliensis, L. donovani* および *Trypanosoma cruzi* のハムスターとマウス体内での運命 ——予報——

橋口　義久[1]・古谷　正人[2]・岡村　宜典[1]

宿主動物と *Leishmania braziliensis* および *L. donovani* との相互関係を *in vivo* で解析する一つの試みとして，上記原虫を diffusion chamber に封入，ハムスターおよびマウスの皮下，腹腔，陰嚢（ハムスターのみ）へ外科的に移植，継時的に chamber を回収し，まず，それらの世代転換（transformation）や生存を調べた。また比較のため，*Trypanosoma cruzi* についても同様な実験を試みた。その結果，2種の *Leishmania* はハムスターおよびマウスの皮下，腹腔内では，極めて短期間の生存を示したが，ハムスターの陰嚢内 chamber ではより長時間生存した（図1，2）。しかし，いずれの場合にも promastigote から amastigote への転換は認められず，原虫は短鞭毛・小形の移行型に止まった。一方，*T. cruzi* はハムスターでは生存が短いものの，マウスでは腹腔と皮下の両移植部位において5日以上にわたって生存し，epimastigote から trypomastigote, amastigote への転換も認められた（図3）。以上の結果は，*Leishmania* 属と *Trypanosoma* 属間での世代転換が極めて異なること，また両属原虫の生存期間は，diffusion chamber 内という特殊な条件下においても，宿主動物の種によって異なることが示唆された。

1　高知医科大学寄生虫学教室　　2 高知医科大学動物実験施設

Japan. J. Trop. Med. Hyg., Vol. 15, No. 2, 1987, pp. 105–116

CLINICAL TRIAL OF TRIMETHOPRIM-SULFAMETHOXAZOLE IN THE TREATMENT OF VIVAX, OVALE AND FALCIPARUM MALARIA[1]

YUKIO YOSHIDA[2], YOSHITSUGU MATSUMOTO[2], MINORU YAMADA[2], TSUNEZO SHIOTA[2], HISAO YOSHIKAWA[2], KAE OKABAYASHI[2], TATSUYA TEGOSHI[2], TETSUYA YOSHIKAWA[2], AKIO KOBAYASHI[3] AND RYUKI KIN[3]

Received January 22 1987/Accepted March 30 1987

Abstract: A compound of trimethoprim (TMP) and sulfamethoxazole (SMZ) was effective in the treatment of vivax, ovale and falciparum malaria. Two patients with vivax malaria received 16 mg TMP/80 mg SMZ/kg body weight in 3 divided doses daily for 3 days. A patient with ovale malaria received 10 mg TMP/50 mg SMZ/kg body weight in 4 divided doses daily for 4 days. These doses were followed by 15 mg primaquine daily for 2 weeks. A patient with falciparum malaria received 16 mg TMP/80 mg SMZ/kg body weight in 3 divided doses daily for 3 days. In all patients, radical cures were achieved; neither relapse nor recrudescence has occurred. Trimethoprim-sulfamethoxazole succeeded in causing rapid clearance of asexual-parasites from peripheral blood of the patients and producing rapid defervescence. No symptomatic adverse reactions to TMP-SMZ were experienced.

INTRODUCTION

In Japan, indigenous malaria (falciparum, vivax and malarial malaria) was eradicated by the 1960s. However, the problem of malaria imported from other parts of the world has become more serious since 1970. During 1972–1981, 697 imported cases of malaria, including 15 deaths in falciparum malaria, were reported in Japan (Ohtomo and Hioki, 1985). Most of the standard anti-malarial drugs including amodiaquine, chloroquine, proguanil, primaquine and pyrimethamine are not commercially available in Japan. Physicians are anticipating anti-malarial drugs which can be easily obtained in Japan.

The effectiveness of trimethoprim-sulfamethoxazole (TMP-SMZ), which is commercially available in Japan, in the treatment of falciparum and vivax malaria has been reported. However, no study has been done on the efficacy of TMP-SMZ in treating ovale malaria. The present paper describes satisfactory therapeutic results of this compound in the treatment of symptomatic vivax, ovale and falciparum malaria.

1 Contribution No. 562 from the Department of Medical Zoology, Kyoto Prefectural University of Medicine
2 Department of Medical Zoology, Kyoto Prefectural University of Medicine, Kyoto 602, Japan
3 Department of Communicable Disease, Kyoto Municipal Hospital, Kyoto 604, Japan

Methods

The cases in the present study include 2 vivax malaria patients (Case 1 and 2), one ovale malaria patient (3) and one falciparum malaria patient (4). All patients were hospitalized and examined as follows: 1) Thin films of peripheral blood were examined for identification of species and stages of malarial parasite and for parasite counts. The number of parasites/200 white blood cells were converted into the number of parasites per μl (parasite count) by calculations based on white blood cell counts taken on the same days. 2) Axillary temperature was taken. 3) Routine hemograms were done, recording the number of red blood cells/μl (RBC), the ratio of reticulocyte to whole RBC (Retics), hemoglobin amount (Hb), hematocrit (Ht), the number of platelets/μl (Plts), the number of white blood cells/μl (WBC) and differential white blood cells count. 4) Liver function tests and other routine biochemistry were performed.

All patients were treated with an oral TMP-SMZ compound (Baktar®, Shionogi & Co., LTD., Osaka, Japan). One tablet contained 80 mg TMP and 400 mg SMZ. Cases 1, 2 and 4 were administered 16 mg TMP/80 mg SMZ/kg body weight (Bw) in 3 divided doses daily for 3 days. Case 3 was administered 10 mg TMP/50 mg SMZ/kg Bw in 4 divided doses daily for 4 days. The casualties totaled 12×3 tablets in Case 1 (Bw=60 kg), 8×3 tablets in Case 2 (Bw=40 kg), 9×4 tablets in Case 3 (Bw=72 kg) and 14×3 tablets in Case 4 (Bw=70 kg). In addition, Cases 1, 2 and 3 were administered 15 mg primaquine daily for 14 days following the TMP-SMZ doses.

Sera from Cases 2 and 4 were analyzed for the presence of antibodies to erythrocytic stages of *Plasmodium vivax* and *P. falciparum* by an indirect fluorescent antibody technique (IFA).

Case Study

Figures 1–4 are the courses of treatment showing body temperature, asexual-parasite counts and sexual-parasitemia of the patients.

Case 1 (Vivax malaria with low parasitemia) (Figure 1)

Present illness: On 2 September 1981, a 33-year-old Japanese male developed chills and fever and visited the Department of Medical Zoology, Kyoto Prefectural University of Medicine for consultation. Immediate blood examination revealed the presence of *P. vivax*, and parasite count was recorded as 675 parasites/μl of blood with 89% trophozoites and 11% schizonts. On 3 September he was hospitalized. Tertian-type high fever attack was found on 4 (39.7°C) and 6 (39.8°C) September and another high parasite count (675/μl) was recorded on 5 September. A hemogram on 4 September recorded Hb of 14.3 g/d*l*; Ht 45.8%; RBC 461×10^4; WBC 5,000 with 40% neutrophils, 17% lymphocytes, 9% monocytes, 33% eosinophils and 1% basophils. On 7 September, Hb was 12.7 g/d*l*, Ht 40.0%, RBC 401×10^4 and WBC 4,100. A physical examination showed a slight hepato-splenomegaly.

History: The patient suffered his first attack of malaria in Zaire in March 1975. He had been there since September 1974 doing anthropological investigations. Until February 1975, one month prior to the onset of the first attack, he had continued weekly chloroquine. This attack was terminated with chloroquine (dose unknown). He returned to Japan in September 1975. During his next visit to Zaire from March 1978 to March 1979, the patient suffered another malarial attack, which subsided after treatment in a hospital. He made a third visit to

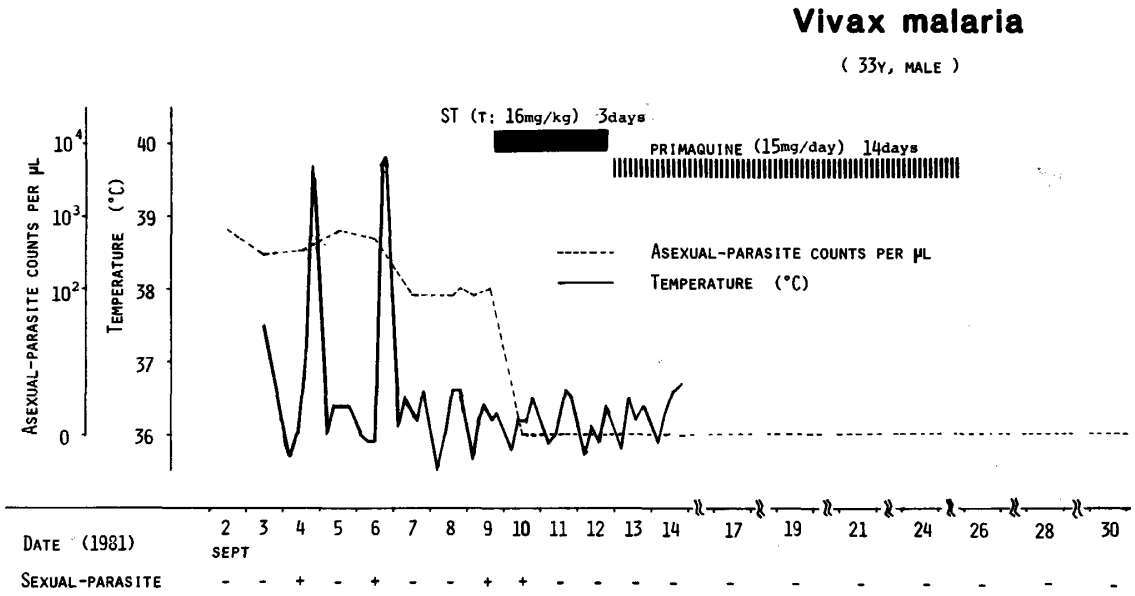


Figure 1 Course of temperature, asexual-parasite count and sexual-parasitemia in the patient, a 33-year-old Japanese male with vivax malaria (Case 1). The patient was given an oral compound of trimethoprim-sulfamethoxazole (ST) at a dosage of 16 mg TMP/80 mg SMZ/kg Bw in 3 divided doses daily for 3 days, followed by 15 mg primaquine daily for 14 days.

Zaire, from August 1979 to February 1980. During this period no further attack was experienced. On 5 June 1980, 3 months after his return from Zaire to Japan, he developed fever and chills and took chloroquine phosphate Resochin® (dose unknown), without successive primaquine administration. After that the patient had no suggestion of malaria for 15 months until the present episode of illness.

Treatment: On 9 September 1981, he was started on 12 oral tablets of TMP-SMZ in 3 divided doses daily (16 mg TMP/80 mg SMZ/kg Bw/a day) for 3 days. Beginning 13 September, 15 mg primaquine was administered daily for 14 days.

An examination, done just prior to this treatment, revealed a parasite count of 82/μl of blood with 100% asexual-parasite. Asexual-parasite count became 0/μl within 24 hours after the first dose was given and remained at 0/μl after this time. Sexual-parasite count was 0/μl 52 hours after the beginning of treatment. Since then no parasites were observed on thin films. The patient's temperature was normal since 7 September. A hemogram on 11 September recorded Hb of 13.4 g/dl; Ht 38.6%; RBC 421×10^4; WBC 5,200 with 47% neutrophils, 30% lymphocytes, 3% monocytes, 19% eosinophils and 1% basophils. The results of the following examinations on 21, 24, 28, 29 September were within normal range, except for eosinophilia. Upon later review of the smears, microfilaria was detected and identified *Dipetalonema perstans* as previously reported (Yoshida *et al.*, 1983). Eosinophilia recorded throughout the period examined might be due to *D. perstans* infection. No symptomatic adverse reactions to TMP-SMZ were observed. To date, no relapse of malaria has been experienced.

Case 2 (Vivax malaria with high parasitemia) (Figure 2)

Present illness: On 7 June 1984, a 37-year-old Japanese nurse developed chills and fever.

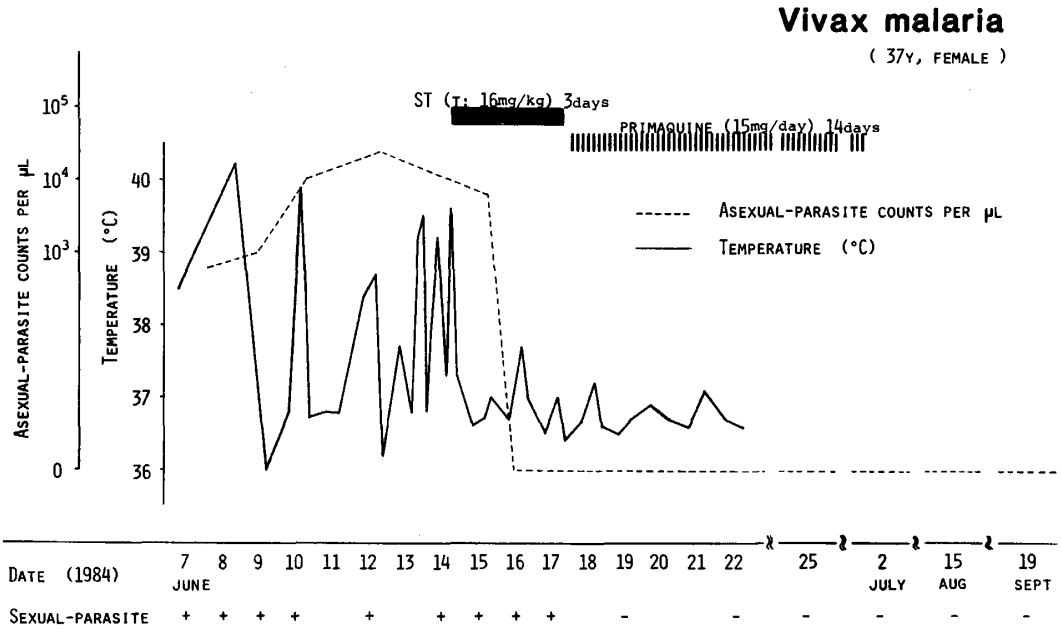


Figure 2 Course of temperature, asexual-parasite count and sexual-parasitemia in the patient, a 37-year-old Japanese female with vivax malaria (Case 2). The patient was given an oral compound of trimethoprim-sulfamethoxazole (ST) at a dosage of 16 mg TMP/80 mg SMZ/kg Bw in 3 divided doses daily for 3 days, followed by 15 mg primaquine daily for 14 days.

On 8 June she made a blood smear and found a malaria parasite. On 9 June she was admitted to Kyoto Municipipal Hospital and the maralial parasite was identified as *P. vivax*. There were 688 parasites/μl on 8 June and 1,075/μl on 9 June; asexual-parasites were 97% and 98% of total parasites respectively. A hemogram on 9 June recorded Hb of 13.6 g/d*l*; Ht 40.1%; RBC 427×10^4; WBC 4,300 with 26% neutrophils, 63% lymphocytes, 10% monocytes and 1% basophils. Liver function tests and other routine biochemistry were all normal. The highest parasite count was 25,870/μl with 93.9% trophozoites, 0.5% schizonts and 5.6% gametocytes on 12 June. Intermittent tertian paroxysms peaking at the night on 8, 10 and 12 June were marked, although on 13 and 14 June paroxysms were irregular. The highest temperature recorded was 40.2°C on 8 June.

History: The patient had traveled in Thailand, Malaysia, Nepal, India and Australia 4 times in 10 years; October 1975 to November 1976, February 1978 to November 1979, February 1980 to July 1980 and October 1981 to September 1983. She took a prophylactic drug against maralia during the first trip only. She suffered her first attack of malaria in India in May 1980. This attack subsided after taking a course of amodiaquine (Camoquin®, dose unknown). In August 1980, one month after she returned to Japan, a relapse was experienced, which was terminated with amodiaquine (Camoquin®, dose unknown). This was not followed by primaquine. In November 1980 she had a normal delivery of a girl. Until 7 June 1984, no replapse occurred.

Treatment: From 14 June 1984 in the present therapeutic schedule, she was given 8 oral tablets of TMP-SMZ in 3 divided doses daily (16 mg TMP/80 mg SMZ/kg Bw/a day) for 3 days followed by 15 mg primaquine daily for 14 consecutive days.

On 14 June an examination done just prior to this treatment, revealed a parasite count of 12,765/μl with 68% trophozoites, 7.9% schizonts and 24.0% gametocytes. A hemogram on this day recorded Ht 35.0%, RBC 356×10^4 and WBC 3,000. Asexual-parasite count became 0/μl within 48 hours after the beginning of treatment. Thereafter the temperature was normal although a slight fever (37.7°C) on 16 June was experienced. A hemogram on 16 June recorded Hb of 9.8 g/dl; Ht 31.0%; RBC 312×10^4; Retics 1.3%; Plts 66×10^3; WBC 6,700 with 57% neutrophils, 31% lymphocytes, 11% monocytes and 1% eosinophils. On 19 June the recordings were Ht 28.0%, RBC 308×10^4 and WBC 4,900. Since then, the Hb and Ht levels gradually returned to normal over a period of 2 months. The number of red blood cells/μl was 341×10^4 on 26 June, 328×10^4 on 2 July, 397×10^4 on 15 August and 438×10^4 on 19 September. Hematocrit was 30.5%, 32.0%, 38.0% and 41.0% respectively. Hemoglobin amount was 10.4 g/dl on 26 June, 12.7 g/dl on 15 August, and 13.6 g/dl on 19 September. Reticulocytes was 8.0% and Plts was 291×10^3 on 26 June. Liver function tests and other routine biochemistry on 26 June were all normal. No symptomatic adverse reactions to TMP-SMZ were observed. To date, no relapse has been experienced.

Serum was analyzed for antibodies against malarial parasites by the IFA test; the serum dilution end-points were 1:256 against *P. vivax* and 1:64 against *P. falciparum* on 12 June and 2 July. On 15 August these were 1:64 against *P. vivax* and 1:16 against *P. falciparum.*

Case 3 (Ovale malaria) (Figure 3)

Present illness: A 29-year-old Japanese male developed fever and chills on 29 May and 2 June 1983, 3 months after his return to Japan from Kenya. On 2 June he was admitted to Kyoto Municipal Hospital when he was temporarily diagnosed as vivax malaria. However, upon later

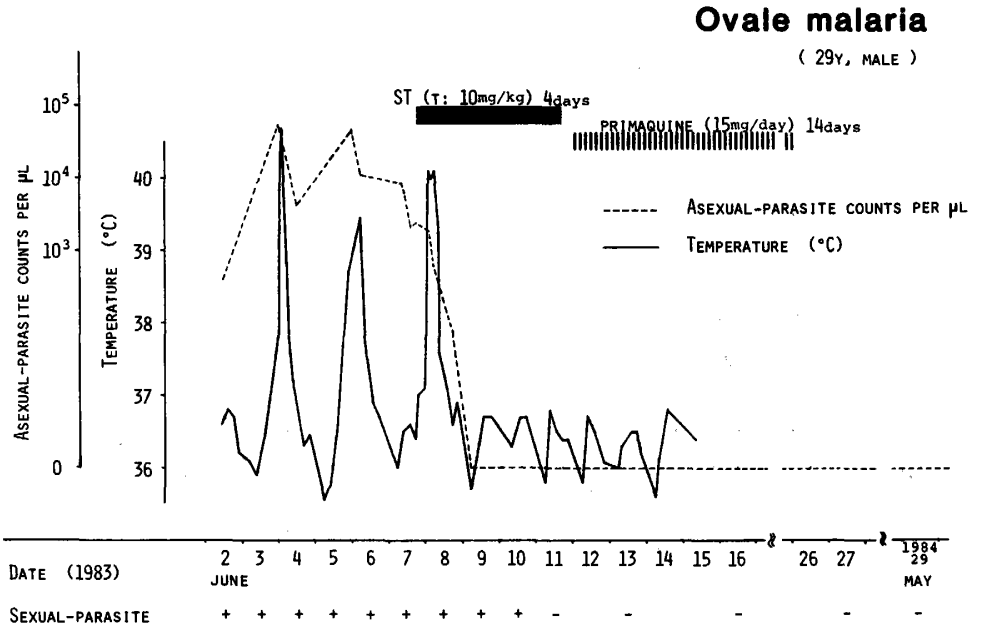


Figure 3 Course of temperature, asexual-parasite count and sexual-parasitemia in the patient, a 29-year-old Japanese male with ovale malaria (Case 3). The patient was given an oral compound of trimethoprim-sulfamethoxazole (ST) at a dosage of 10 mg TMP/50 mg SMZ/kg Bw in 4 divided doses daily for 4 days, followed by 15 mg primaquine daily for 14 days.

review of the smears the parasites were identified as *P. ovale* as previously reported (Matsumoto *et al.*, 1986). There were 200 parasites/μl with 95% trophozoites and 5% gametocytes. A hemogram on 2 June recorded Hb of 15.4 g/d*l*; Ht 46.1%; Plts 33.1×10^3; RBC 458×10^4; WBC 4,000 with 47% neutrophils, 24% lymphocytes, 27% monocytes and 2% eosinophils. On 4 June the highest parasite count (53,648/μl with 99.2% trophozoites, 0.4% schizonts and 0.4% gametocytes) and the highest temperature (40.7°C) was recorded. A hemogram on 6 June recorded Hb of 13.4 g/d*l*; Ht 40.1%; Plts 97.0×10^3; RBC 445×10^4; WBC 6,400 with 84% neutrophils, 9% lymphocytes and 6% monocytes. Intermittent tertain paroxysms peaking at midnight became marked. Liver function tests and other routine biochemistry which were done on 3 and 6 June were almost normal except for total bilirubin (1.7 mg/d*l* on 3 June and 2.3 mg/d*l* on 6 June), cholesterol (102 mg/d*l* on 6 June) and total protein (6.2 g/d*l* on 3 June and 5.8 g/d*l* on 6 June). A physical examination on 6 June showed moderate enlargement of the spleen and the liver.

History: The patient had stayed in Kenya from June 1978 to March 1979, July 1980 to February 1981, August 1981 to October 1981 and July 1982 to the end of February 1983. Most of his time was spent in the Turkana district doing anthropological investigations. During his first visit, he irregularly took chloroquine for prophylaxis but he suffered from a malaria-like attack and was cured with sulfamonomethoxine. During his following stays in Kenya, he regularly took a prophylactic drug (chloroquine) against malaria. Until 29 May 1983, no further malarial attacks had been experienced.

Treatment: On 7 June 1983, he was started on 9 oral tablets of TMP-SMZ in 4 divided doses daily (10 mg TMP/50 mg SMZ/kg Bw/a day) for 4 days. Beginning 12 June, 15 mg primaquine was given daily for 14 days.

An examination on 7 June, done just prior to this treatment, revealed a parasite count of 2,993/μl of blood with 79.5% trophozoites and 20.5% gametocytes. Asexual-parasite count became 0/μl within 36 hours after the first dose was given and disappeared after this time. Sexual-parasite count became 0/μl 3.5 days after the beginning of treatment. Since then no parasites were observed on thin films. At midnight on the first day of treatment the temperature was 40.1°C. After that the temperature became normal. Daily hemograms revealed anemia, with the Hb gradually falling to 10.6 g/d*l* on 11 June. A hemogram on the same day recorded Ht 33.8%, RBC 345×10^4, Retics 2.1%, Plts 116×10^3 and WBC 4,800. After that the hemogram gradually returned to normal. A hemogram on 27 June recorded Hb of 14.0 g/d*l*; Ht 41.9%; RBC 465×10^4; Plts 187×10^3; WBC 4,900 with 54% neutrophils, 26% lymphocytes, 13% monocytes, 5% eosinophils and 1% basophils. Liver function tests and other routine biochemistry were all normal. No parasites were observed on thin films. No symptomatic adverse reactions to TMP-SMZ were observed. A hemogram on 29 May 1984, just one year after the onset of the illness, recorded Hb of 17.2 g/d*l*; Ht 51.5%; RBC 521×10^4; Plts 175×10^3; WBC 6,700 with 63% neutrophils, 24% lymphocytes, 8% monocytes, 4% eosinophils and 1% basophils. To date, no relapse has been experienced.

Case 4 (Falciparum malaria) (Figure 4)

Present illness: A 48-year-old Tanzanian male developed fever on 5 August 1984 and visited the Department of Medical Zoology, Kyoto Prefectural University of Medicine on 9 August for consultation. He was diagnosed as falciparum malaria and immediately hospitalized in Kyoto Municipal Hospital. Parasite count was 4,371/μl of blood with 99.3% trophozoites and

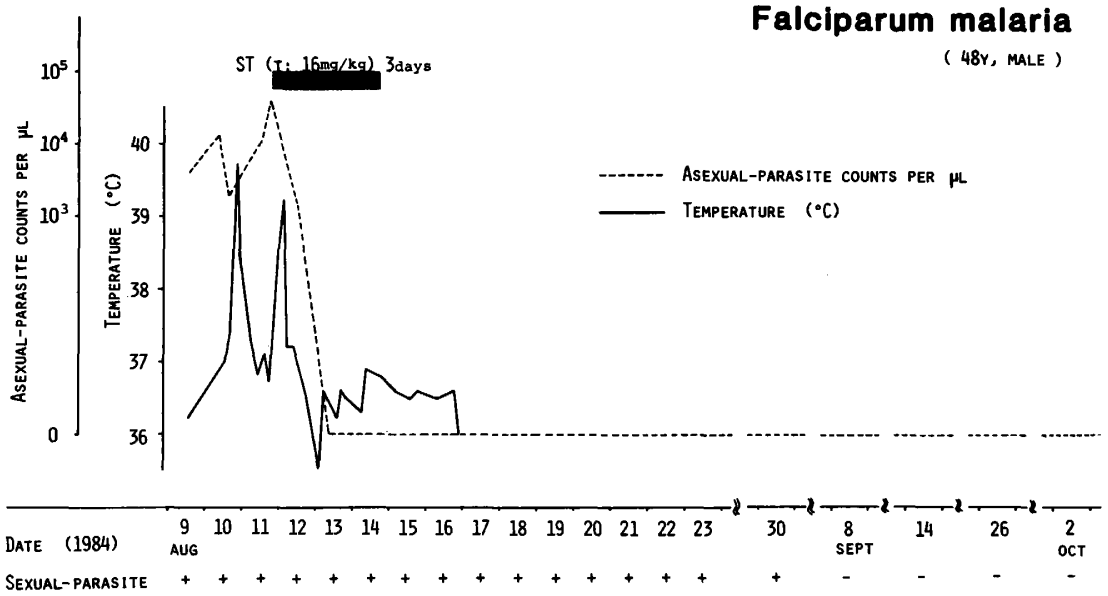


Figure 4 Course of temperature, asexual-parasite count and sexual-parasitemia in the patient, a 48-year-old Tanzanian male with falciparum malaria (Case 4). The patient was given an oral compound of trimethoprim-sulfamethoxazole (ST) at a dosage of 16 mg TMP/80 mg SMZ/kg Bw in 3 divided doses daily for 3 days.

0.7% gametocytes. The highest parasite count (21,476/μl) was recorded on 11 August and the highest temperature (39.7°C) was recorded on 10 August. A hemogram on 10 August recorded Hb of 14.2 g/dl; Ht 42.9%; RBC 501×10^4; Plts 243×10^3; WBC 5,600 with 53% neutrophils, 40% lymphocytes, 3% monocytes and 5% eosinophils. Liver function tests and other routine biochemisty which were done on 10 August were almost normal except for triglyceride (208 mg/dl), LDH (260 U/l) and CPK (125 U/l).

History: The patient was born and raised in Tanzania and did not leave the country until 1959. He stayed in the U.S.A. from 1959 to 1976. After he had returned to Tanzania, he suffered an attack of malaria (date unknown). He arrived in Japan on 30 April 1984. The next day, he developed malarial symptoms (fever and headache). From that time until 5 August, he experienced 7 attacks at two-week intervals. In early June he took a total dose of 2 g chloroquine sulphate (Nivaquine®) over 4 days. The fever disappeared but the headache remained. On 15 July, he took 400 mg amodiaquine (Camoquin®) daily for 3 days. After that he took 400 mg amodiaquine once a week until 5 August.

Treatment: Beginning 11 August 1984, he was started on 14 oral tablets of TMP-SMZ in 3 divided doses daily (16 mg TMP/80 mg SMZ/kg Bw/a day) for 3 days.

An examination on 11 August, done just prior to this treatment, revealed a parasite count of 20,440/μl with 99.9% trophozoites and 0.1% gametocytes. Asexual-parasites disappeared within 40 hours after the first dose was given, but sexual-parasites remained for more than 20 days. On the first day of treatment, the temperature was 39.2°C but after that returned to normal. A hemogram on 13 August, 40 hours after the beginning of treatment, recorded Hb of 13.6 g/dl; Ht 40.2%; RBC 472×10^4; Plts 172×10^3; WBC 4,900 with 42% neutrophils, 43% lymphocytes, 6% monocytes and 9% eosinophils. Blood examination was done on 17, 23, 28

and 30 August; 8, 14 and 26 September; and 2 October. Hb ranged from 14.7 g/d*l* on 28 August to 15.8 g/d*l* on 2 October; Ht ranged from 42.5% on 23 August to 45.5% on 14 September and 2 October; RBC ranged from 486×10^4 on 23 August to 536×10^4 on 14 September. Liver function tests and other routine biochemistry were almost normal except for CPK (136 U/*l*). A slight eosinophilia (8–12%) persisted throughout the examination period. This was considered to be due to concurrent *Necator americanus* infection. No symptomatic adverse reactions to TMP-SMZ were observed. No recrudescence has been experienced until April 1985, when the patient returned to Tanzania.

Serum was analyzed for antibodies against malarial parasites by the IFA test; the serum dilution end-points were 1:1,024 on 10 August and 1:256 on 17, 24 and 30 August against *P. falciparum* and 1:64 on 17 August and 1:16 on 10, 24 and 30 August against *P. vivax*.

Discussion

Trimethoprim-sulfamethoxazole (TMP-SMZ) in a fixed-ratio compound (TMP : SMZ=1 : 5), sometimes designated co-trimoxazole, is listed as an alternate choice for treatment of infections due to a variety of microorganisms. One normal strength tablet usually contains 80 mg TMP and 400 mg SMZ. This has been marketed under various trade names. The usual recommended dose for adults is 2 tablets twice daily.

Trimethoprim (2, 4-diamino-5-[3, 4, 5-trimethoxybenzyl] pyrimidine), one of the pyrimidine derivatives, is a potent inhibitor of the enzyme dihydrofolate reductase (DHFR) in microorganisms and interferes competitively with the conversion of dihydrofolic (folic) acid to tetrahydrofolic (folinic) acid. Trimethoprim has a particularly high affinity for bacterial DHFR but binds less tightly to the corresponding mammalian enzyme. Sulfamethoxazole (3-[*p*-amino-phenylsulfonamido]-5-methylisoxazole), a semi-long-acting sulfonamid, is a structual analog of *p*-aminobenzoic acid. Sulfamethoxazole competitively inhibits the utilization of *p*-aminobenzoic acid in the synthesis of dihydrofolic acid (folic acid) by microorganisms (Salter, 1982; Wormser and Keusch, 1979). In vitro, these agents are shown to be decidedly more active together than either drug is alone because of sequential blockade of the folate pathway. The efficacy of TMP-SMZ in toxoplasmosis (Grossman *et al.*, 1978), leishmaniasis (Kandil, 1973), *Pneumocystis* pneumonia (Hughes *et al.*, 1975; Harris *et al.*, 1980), and malaria has been demonstrated by various researchers (Salter, 1982).

For treatment of vivax malaria, there are only 2 studies on imported cases in Japan by Yoshioka and Kosaka (1978) and Yamaguchi *et al.* (1986), and on resident populations of endemic areas by Minhas and Siddiq (1971) in Pakistan and by Lal (1982) in Punjab, India. Using a standared daily-dosage of 4 tablets of TMP-SMZ for 5 days on adult patients, Minhas and Siddiq demonstrated that fever resolved within 48 hours and parasitemia disappeared in a mean of 1.8 days. Lal compared the responses of parasitemia and fever in resident children with vivax malaria to standerd doses of chloroquine and to different dosage schedules of TMP-SMZ. His study showed that chloroquine produced the most rapid clearance, followed by TMP-SMZ in high daily-dose schedules (8 tablets/day for 2 days or the same dosage followed by 4 tablets/day for 3 days), and then TMP-SMZ in standard dose schedules (4 tablets/day for 3 or 5 days). In Japan, Yoshioka and Kosaka (1978) used 4 tablets/day of TMP-SMZ for 7 days, and Yamaguchi *et al.* (1986) used 4–8 tablets/day for 1–4 days, both with successful elimination of the parasite from the blood. In our cases of imported vivax malaria, Case 1 was given 12 tablets/day (16 mg

TMP/80 mg SMZ/kg Bw/day) for 3 days and Case 2 was given 8 tablets/day (16 mg TMP/80 mg SMZ/kg Bw/day) for 3 days, then both were followed by 15 mg primaquine daily for 14 days. This schedule succeeded in producing radical cures. Asexual-parasites disappeared from the blood of Case 1 in 24 hours and of Case 2 in 48 hours.

Until this study, no studies of the effects of TMP-SMZ on ovale malaria had been published. Nine tablets/day (10 mg TMP/50 mg SMZ/kg Bw/day) for 4 days followed by 15 mg primaquine daily for 14 days succeeded in producing a radical cure. Asexual-parasitemia disappeared within 36 hours and fever resolved within 24 hours.

The efficacy of co-trimoxazole in falciparum malaria has been demonstrated by various researchers (Benjapongs *et al.*, 1970; Wolfensberger, 1970; Fasan, 1971; Yamaguchi *et al.*, 1986). Benjapongs *et al.* (1970) gave TMP-SMZ (4 tablets daily for 2.5–10 days) to previously-untreated acute falciparum malaria and previously-treated chloroquine-resistant malaria. With the 2.5 day schedule, results were unsatisfactory in both groups. With 4 tablets daily, fever resolved in an average of 3 days and asexual-parasites disappeared from blood in 4.3 days. They concluded that, to get the best therapeutic results, treatment should be continued at least 2 days after the disappearance of parasites from blood; that is, the total dosage required to achieve a "complete effect" was 30 to 40 tablets. The clinical response to the same dose was somewhat faster in patients with previously-treated chloroquine-resistant falciparum malaria. Wolfensberger (1970) used 8 tablets daily for 3–4 days in patients with acute falciparum malaria; the TMP-SMZ compared favorably with chloroquine. Fasan (1971) used a single dose of TMP-SMZ in falciparum malaria in semi-immune Nigerian schoolchildren aged 5–12 years. The dose (8 mg TMP/40 mg SMZ/kg Bw) was found to be effective. Fasan also found that half dosages of the same compound were effective. Yamaguchi *et al.* (1986) treated 2 imported cases of falciparum malaria in Japan, one with TMP-SMZ 4 tablets 3 times a day followed by chloroquine diphosphate 600–450 mg/day for 4 days, and the other with TMP-SMZ 3–8 tablets/day for 4 days. They both recovered completely. Falciparum malaria, Case 4 of our study, was presumed to be somewhat resistant to amodiaquine; however, it responded well to TMP-SMZ at a dosage of 16 mg TMP/80 mg SMZ/kg Bw/day for 3 days. Asexual-parasitemia disappeared within 40 hours and fever resolved within 24 hours.

The only study on malarial malaria has been done by Fasan (1971). However, their patients had mixed infections of *P. falciparum* and *P. malariae.* He noticed that there was a reappearance of quartan parasites 3 days after single doses of TMP-SMZ(4 mg TMP/20 mg SMG/kg Bw). He proposed 2 possible reasons. One is that the individual parasites noted were gametocytes, which in the case of *P. malariae* were difficult to differentiate in a thick film. The other is that TMP-SMZ at this low dosage was less effective against quartan malaria parasites.

In the treatment of malaria, TMP-SMZ has been considered to be a rapidly acting schizonticide. It has been noted that *P. falicparum* crescents were detected as late as one to 2 weeks after treatment with TMP-SMZ, otherwise the response was good. Benjapongs *et al.* (1970), concluded that TMP-SMZ had no effect on gametocytes of *P. falciparum* and therefore primaquine should be prescribed for malaria patients with TMP-SMZ to prevent transmission. In our observation, *P. falciparum* gametocytes were not cleared from the blood of a patient who received TMP-SMZ until 3 weeks following treatment, otherwise the response was good. Gametocytes of *P. vivax* and *P. ovale,* however, might be cleared from the blood of patients by TMP-SMZ, because they disappeared before starting administration of primaquine.

The combination is generally well tolerated even with a large dose of long term administration, as seen in treatment of *Pneumocystis* infection (20 mg TMP/100 mg SMZ/kg Bw/a day for 14 days) (Hughes *et al.*, 1975) or prophylaxis against it in children (4 mg TMP/20 mg SMZ/kg Bw/a day over months) (Harris *et al.*, 1980). The most common adverse reactions are mild gastrointestinal symptoms including nausea, vomiting, and less frequently diarrhea, cramps, glossitis, stomatitis, or jaundice (Salter, 1982; Wormser and Keusch, 1979). Regarding the use of TMP-SMZ for malaria, Benjapongs *et al.* (1970) reported slight nausea and vomiting (in 2 of 5 patients) during the first few days on the 3-tablets-daily schedule and noted one case of mild transient leukopenia. Lal (1982) reported vomiting was noted in 13 of 98 cases, although vomiting was notably common with chloroquine. In our study, no such adverse reaction to TMP-SMZ was observed.

A number of hematologic abnormalities including thrombocytopenia, leukopenia and anemia (macrocytic, megaloblastic, hemolytic or aplastic) have been described in patients receiving TMP-SMZ but they are rare (Salter, 1982; Wormser and Keusch, 1979). Patients with known folic acid or vitamin B_{12} deficiency are at increased risk from the antifolic effects of the drugs, and patients with questionably adequate folic-acid stores, such as pregnant women, the elderly, patients with malabsorption or malnutrition, or those with chronic hemolysis (such as sickle-cell disease), should be carefully observed. In this study, all patients showed anemia during the treatment but recovered soon after the treatment. This might be mainly due to malarial infection itself.

In conclusion, our study showed that TMP-SMZ may be a safe choice to produce radical cures for malaria caused by any species of malarial parasites, at least vivax, ovale and falciparum malaria at the dosage described above. Trimethoprim-sulfamethoxazole should be examined further in the treatment of chloquine-resistant malaria and in those countries in which antimalarial drugs are not easy to obtain.

Acknowledgements

We are indebted to Professor Mamoru Suzuki of the Department of Parasitology, Gunma University, School of Medicine for performing the serological tests of Case 2 and 4 in this study.

References

1) Benjapongs, W., Sadudi, N. and Noeypatimanond, S. (1970): Trimethoprim/sulphamethoxazole combination in the treatment of falciparum malaria, J. Med. Ass. Thailand, 53, 849–856
2) Fasan, P. O. (1971): Trimethoprim plus sulphamethoxazole compared with chloroquine in the treatment and suppression of malaria in African school-children, Ann. Trop. Med. Parasitol., 65, 117–121
3) Grossman, P. L., Krahenbuhl, J. L. and Remington, J. S. (1978): In vivo and in vitro effects of trimethoprim and sulfamethoxazole on *Toxoplasma* infection, In: Current chemotherapy, Siegenthaler, W. and Lüthy, R. ed., 1, 135–137, American Society for Microbiology, Washington, D. C.
4) Harris, R. E., McCallister, J. A., Allen, S. A., Barton, A. S. and Baehner, R. L. (1980): Prevention of Pneumocystis pneumonia, Am. J. Dis. Child., 134, 35–38
5) Hughes, W. T., Feldman, S. and Sanyal, S. K. (1975): Treatment of *Pneumocystis carinii* pneumonitis with trimethoprim-sulfamethoxazole, Can. Med. Ass. J., 112, 47–50
6) Kandil, E. (1973): Treatment of cutaneous leishmaniasis with trimethoprim-sulfamethoxazole combination, Dermatologica, 146, 303–309

7) Lal, H. (1982): A comparative trial of oral chloroquine and oral co-trimoxazole in vivax malaria, Am. J. Trop. Med. Hyg., 31, 438–440
8) Matsumoto, Y., Matsuda, S. and Yoshida, Y. (1986): Ultrastructure of erythrocytic stages of *Plasmodium ovale* in humans, Am. J. Trop. Med. Hyg., 35, 689–696
9) Minhas, A. L. and Siddiq, K. Y. (1971): Trimethoprim-sulphamethoxazole (Septran) in *P. falciparum* and *P. vivax* malaria, Medicus (Karachi), 42, 310–314
10) Ohtomo, H. and Hioki, A. (1985): Disseminated intravascular coagulation in malaria, Modern Media, 31, 159–168
11) Salter, A. J. (1982): Trimethoprim-sulfamethoxazole: An assessment of more than 12 years of use, Rev. Infect. Dis., 4, 196–224
12) Wolfensberger, H. R. (1970): Clinical trials with 'Fansidar', 'Bactrim', and chloroquine in 300 cases of acute falciparum malaria, Far East Med. J., 6, 48–50
13) Wormser, G. P. and Keusch, G. T. (1979): Trimethoprim-sulfamethoxazole in the United States, Ann. Intern. Med., 91, 420–429
14) Yamaguchi, K., Koga, H., Kohno, S., Shigeno, Y., Suzuyama, Y., Hirota, M., Saito, A., Hara, K., Amagasaki, T., Iwanaga, S., Okuno, K., Fujiwara, T., Murata, I., Shinzato, K., Tsutsumi, T., Goto, J. and Ishizaki, T. (1986): Clinical evaluation of SMX/TMP on treatment of the patients with malaria, J. Jpn. Ass. Infect. Dis., 60, 1027–1035
15) Yoshida, Y., Shiota, T., Yamada, M., Matsumoto, Y., Okabayashi, K., Yoshikawa, H., Takino, T., Nakagawa, Y. and Yokota, S. (1983): Report of an imported case of *Dipetalonema perstans* in Japan, Southeast Asian J. Trop. Med. Pub. Hlth., 14, 341–344
16) Yoshioka, M. and Kosaka, S. (1978): A recent case of malaria with reference to its present status, Med. J. Aomori Pref. Central Hospital, 23, 81–85

トリメトプリム・サルファメトキサゾールによる三日熱，卵型および熱帯熱マラリア症例の治療成績

吉田　幸雄[1]・松本　芳嗣[1]・山田　稔[1]・塩田　恒三[1]・吉川　尚男[1]
岡林　加枝[1]・手越　達也[1]・吉川　哲也[1]
小林　祥男[2]・金　龍起[2]

わが国における輸入マラリア症例は年々，増加の傾向にあるが，現在世界で用いられている抗マラリア剤の殆どのものが市販されていない。ところがわが国でも市販されているトリメトプリム（TMP）とサルファメトキサゾール（SMZ）の合剤が三日熱および熱帯熱マラリアに有効なことが知られてきた。われわれも1981年以来経験した4症例に本剤を用いたところ良好な治療効果を得た。まず2例の三日熱マラリアに対して，16 mg TMP/80 mg SMZ/kg/日を分3し3日間投与し，続いてプリマキン15 mg/日を14日間投与した。1例の卵型マラリアに対しては10 mg TMP/50 mg SMZ/kg/日を分4し4日間投与し，続いてプリマキン上記量を与えた。さらに1例の熱帯熱マラリアに対しては16 mg TMP/80 mg SMZ/kg/日を分3して3日間与え，プリマキンは投与しなかった。いずれの例においても発熱など臨床症状は急速に改善し，治療開始36－52時間後の検血で繁殖体は消失していた。生殖母体は熱帯熱マラリアにおいて治療終了後約10日間検出された後消失したが，他のマラリアでは治療期間中に消失した。これらはその後，再発あるいは再燃を示しておらず，本治療で根治したものと思われる。今回は比較的大量を短期間投与したが本剤によると思われる副作用は認めなかった。本剤により卵型マラリアの治療に成功したのは本報告が最初である。以上の成績から本剤は，特に他の薬剤の入手困難な国に於て，また他の薬剤に耐性を生じたマラリアについて有用であると考える。

1 京都府立医科大学医動物学教室　2 京都市立病院伝染病科

Japan. J. Trop. Med. Hyg., Vol. 15, No. 2, 1987, pp. 117–120

Short Communication

CHAGAS' DISEASE: DELAYED TYPE HYPERSENSITIVITY TO PPD IN RELATION TO SEROREACTIVITY TO *TRYPANOSOMA CRUZI* IN ECUADOR

MASATO KAWABATA[1], TATSUYUKI MIMORI[2], YOSHIHISA HASHIGUCHI[3] AND VICENTA V. DE CORONEL[4]

Received December 18 1986/Accepted April 15 1987

Abstract: In a population from an area endemic for Chagas' disease in Ecuador, cell-mediated immune activity was assessed using delayed type hypersensitivity (DTH) reaction to PPD. Expression of DTH was significantly suppressed in individuals seropositive to *Trypanosoma cruzi,* and the suppression was markedly greater in the age group 10–39 years old than those over 40 years. The results show that cell-mediated immune response is apparently suppressed in patients with Chagas' disease, and tends to be restored in chronic stage. No defference was noted in the serum concentration of immunoglobulins between patients and controls.

INTRODUCTION

It has been well known that infections with several species of parasites are associated with modification of immune response to heterologous antigens. Delayed type hypersensitivity (DTH) skin reactions are used as an effective tool to assess cell-mediated immunity in humans, and have been found to be suppressed in helminthic and protozoal infection (Greenwood *et al.*, 1973; Grove and Frobes, 1979; Kawabata *et al.*, 1983). In patients with Chagas' disease, caused by *Trypanosoma cruzi,* the previous reports on DTH to unrelated heterologous antigens remain controversial. Expression of DTH reactions in acute chagasic patients was observed to be less than that in nonchagasic controls (Teixeira *et al.*, 1978a), whereas no such suppression was found in the chronic patients (Montufar *et al.*, 1977; Corsini *et al.*, 1981).

On the other hand, it has been suggested that *T. cruzi* isolated from various geographic areas may have distinct properties because clinical manifestations show great regional differences. In the present study the expression of DTH to purified protein derivatives (PPD) of tuberculin and the quantification of serum concentration of immunoglobulins (Igs) was performed in a population from an endemic area of Chagas' disease in an attempt to assess their immunological status in Ecuador.

1 Department of Clinical Pathology, Nihon University School of Medicine, Oyaguchi, Itabashi-ku Tokyo 173
2 Department of Parasitic Diseases, Kumamoto University Medical School, Honjo, Kumamoto City, Kumamoto 860
3 Department of Parasitology, Kochi Medical School, Nankoku City, Kochi 781–51
4 Departamento de Parasitologia, Instituto Nacional de Higiene y Medichina Tropical, Apartado 3961, Guayaquil, Ecuador

Correspondence to: Masato Kawabata, M.D.

Materials and Methods

Subjects: The study was conducted in 2 endemic foci for Chagas' disease, Zaruma and surrounding areas (Province of El Oro), and Pedro Carbo and surrouding areas (Province of Guayas). DTH reaction was examined in 255 individuals, while the Ig was quantified in 137 individuals. These subjects were examined for their serum antibodies to *T. cruzi* with a commercially available indirect hemagglutination (IHA)-kit (Hema Chagas, Polychaco, S. A. I. C., Argentina), and classified into 2 groups according to the seroreactivity. IHA titers of 16 or greater were interpreted as positive in this study.

DTH reaction: DTH reaction to intradermal injection of 0.1 m*l* PPD from *Mycobacterium tuberculosis* RT23, equivalent to 0.005 μg of PPD-s, were measured 48 hours later. Induration of 5 mm in diameter or more was considered a positive reaction.

Quantification of serum Ig: The serum concentration of IgG, IgM and IgA were determined by single radial immunodiffusion (SRID) method in agar. The plates were purchased from Medical Biological Laboratories (MBL) Co. Ltd., Japan.

Results and Discussion

Cell-mediated immunity was assessed by testing DTH reactions to PPD in individuals seropositive or seronegative to *T. cruzi*. Positive DTH were significantly less frequent in seropositive individuals than in seronegative individuals (Table 1). When the magnitude of the reaction was analyzed in relation to age among the seropositive group, the DTH was weaker in the 10–39 year age group ($p<0.01$) than in the over 40 year age group ($p<0.05$). The present results suggest that cell-mediated immunity was suppressed in seropositive individuals, but was restored in the older age group, although the difference between 2 groups was not significant. The normalization trend for cell-mediated immunity shows that the proliferative response of lymphocytes from *T. cruzi*-infected mice was suppressed during acute infection, and returned to normal levels during the chronic stage (Hayes and Kierszenbaum, 1981). Humoral immunity remain suppressed since the antibody response to typhoid vaccine or sheep erythrocytes in patients with chronic Chagas' disease remains low (Cunningham *et al.*, 1980; Corsini *et al.*, 1981).

No significant differences in the serum levels of IgG, IgM and IgA were noted between the

Table 1 Delayed type hypersensitivity to PPD in a population from an endemic area of Chagas' disease in Ecuador

Age group	No. of positives[a]/No. of Examined (%)		p[b]
(years)	Seropositives[c]	Seronegatives	
10–39	9/34 (26.5)	64/122 (52.5)	<0.01
40–	22/59 (37.3)	23/40 (57.5)	<0.05

a) reactivity to PPD
b) p values with chi-square test
c) seroreactivity to *T. cruzi* by IHA

Table 2 Serum levels of immunoglobulins in a population from an endemic area of Chagas' disease in Ecuador

	Seropositives[a] (n=96)	Seronegatives (n=41)
IgG	1555.2 (449.2)[b]	1470.9 (433.5)
IgM	139.9 (67.3)	153.0 (73.3)
IgA	240.7 (87.6)	242.9 (100.4)

a) seroreactivity to *T. cruzi* by IHA
b) mean (S.D.), mg/dl

seropositive and seronegative groups (Table 2). The results of our study and previous ones (Lelchuk *et al.*, 1970; Corsini *et al.*, 1981) indicate a normal level of Ig in chagasic patients, but an elevated level of Ig in the acute phase of the infection (Vattuone *et al.*, 1973; Schuñis *et al.*, 1978). Great care, however, must be taken on the fact that the immunological status observed in this study might be restricted to Ecuadorian Chagas' disease caused by different geographical strains of parasites.

Our understanding on the immunopathological implication of immune suppression and its restoration on the clinical course of Chagas' disease is limited. Since DTH reaction mediated by *T. cruzi*-sensitized T-lymphocytes has been shown to be involved in the autoimmune destruction of heart cell *in vitro* (Teixeira *et al.*, 1978b), restoration of cell-mediated immunity might be associated with development of chronic heart lesions. In addition, transition from latent to chronic stage, defined by electrocardioigraphic abnormalities, was found in individuals over 40 years old that were seropositive to *T. cruzi* in Ecuador (Kawabata *et al.*, 1987). To know the precise mechanisms responsible for an evolution of chronic lesions in Chagas' disease, further investigations is needed.

Acknowledgements

We wish to express our appreciations to our collaborators in the Instituto Nacional de Higiene y Medicina Tropical, Guayaquil, Hospital de Zaruma, Provincia de El Oro, and Centro de Salud de Pedro Carbo, Provincia de Guayas, Ecuador.

References

1) Corsini, A. C., Vilela, M. S. and Piedrabuena, A. E. (1981): Chronic Chagas' disease: Unimpaired delayed type hypersensitivity reactions in contrast with humoral suppression to typhoid vaccine, Tropenmed. Parasit., 32, 82–86
2) Cunningham, D. S., Grogl, M. and Kuhn, R. E. (1980): Suppression of antibody responses in humans infected with *Trypanosoma cruzi*, Infect. Immun., 30, 496–499
3) Greenwood, B. M., Whittle, H. C. and Molyneux, D. H. (1973): Immunosuppression in Gambian trypanosomiasis, Trans. R. Soc. Trop. Med. Hyg., 67, 846–850
4) Grove, D. I. and Frobes, I. J. (1979): Immunosuppression in bancroftian filariasis, Trans. R. Soc. Trop. Med. Hyg., 73, 23–26
5) Hayes, M. M. and Kierszenbaum, F. (1981): Experimental Chagas' disease: Kinetics of lymphocyte responses and immunological control of the transition from acute to chronic *Trypanosoma cruzi*

infection, Infect. Immun., 31, 1117–1124
6) Kawabata, M., Izui, S., Anan, S., Kondo, S., Fukumoto, S., Zea, G. F. and Kobayakawa, T. (1983): Circulating immune complexes and their possible relevance to other immunological parameters in Guatemalan onchocersiasis, Int. Arch. Allergy. Appl. Immunol., 72, 128–133
7) Kawabata, M., Uchiyama, T., Mimori, T., Hashiguchi, Y. and Coronel, V. V. (1987): Association of electrocardiographic abnormalities with seropositivity to *Trypanosoma cruzi* in Ecuador, Trans. R. Soc. Trop. Med. Hyg., 81, 7–10
8) Lelchuk, R., Dalmassom, A. P., Inglesini, C. L., Alvarez, M. and Cerisola, J. A. (1970): Immunoglobulin studies in serum of patients with American trypanosomiasis (Chagas' disease), Clin. Exp. Immunol., 6, 547–555
9) Montufar, O. M., Musatti, C. C., Mendes, E. and Mendes, N. F. (1977): Cellular immunity in chronic Chagas' disease, J. Clin. Microbiol., 5, 401–404
10) Schuñis, G. A., Szarfman, A., Coarasa, L. and Vainstok, C. (1978): Immunoglobulin concentration in treated human acute Chagas' disease, Am. J. Trop. Med. Hyg., 27, 473–477
11) Teixeira, A. R., Teixeira, G., Macedo, V. and Prata, A. (1978a): Acquired cell-mediated immunodepression in acute Chagas' disease, J. Clin. Invest., 62, 1132–1141
12) Teixeira, A. R., Teixeira, G., Macedo, V. and Prata, A. (1978b): *Trypanosoma cruzi*-sensitized T-lymphocyte mediated ^{51}Cr release from human heart cells in Chagas' disease, Am. J. Trop. Med. Hyg., 27, 1097–1107
13) Vattuone, N. H., Szarfman, A. and Cappa, S. M. (1973): Antibody response and immunoglobulin levels in humans with acute or chronic *Trypanosoma cruzi* infections (Chagas' disease), J. Trop. Med. Hyg., 76, 45–47

短　報

エクアドル国のシャーガス病流行地住民集団における遅延型皮内反応

川端　真人[1]・三森　龍之[2]・橋口　義久[3]
VICENTA V. DE CORONEL[4]

エクアドル国のシャーガス病患者の細胞性免疫機能を検索する目的で PPD に対する遅延型アレルギー反応を検討した。シャーガス病流行地の住民255名を対象に *T. cruzi* 抗体を IHA test (Hemo chagas, Polychaco SAIC) で検査すると陽性者は93名，陰性者は162名であった。ツベルクリン反応の陽性率は，IHA 陽性者の方が陰性者より有意に低く，特に10－39歳群でその傾向が強かった。この結果からシャーガス病患者では細胞性免疫機能の低下があり，10－39歳群では強い抑制があるが，慢性期になると回復傾向があると推測された。他方，免疫グロブリンを SRID で測定すると IgG, IgM, IgA ともに IHA 陽性者と陰性者の間に差はみられなかった。

1 日本大学医学部臨床病理学教室　2 熊本大学医学部寄生虫病学教室
3 高知医科大学寄生虫学教室　4 エクアドル国立熱帯医学研究所寄生虫部

日本熱帯医学会雑誌　第15巻　第2号　1987　121-137頁

第28回　日本熱帯医学会総会講演抄録（1）

会　期：昭和61年11月22日（土）・23日（日）
会　場：琉球大学医学部講義室
会　長：琉球大学医学部教授　大鶴正満

目　次

幹細胞の変動について
浅見　鳴子，丸山　治彦，大橋　　真，
名和　行文　　(宮崎医大・寄生虫)

4 *Babesia* 感染に対するトキソプラズマ抗原(TLA)投与マウスにおけるT-リンパ球の動態
鈴木　直義，関口　信一，佐藤　基佳，
桜井　治久，斉藤　篤志，広瀬　恒夫
(帯広大・獣医)
尾崎　文雄　　(神戸市)

5 インドネシア・北スマトラのマラリア対策(1) Primaquine 一回投与の *P. f.* gametocyte に対する効果
金子　　明
(JICA 北スマトラプロジェクト，
弘前大・医・寄生虫)
亀井喜世子
(JICA 北スマトラプロジェクト，
帝京大・医・寄生虫)
石井　　明　　(岡山大・医・寄生虫)
鈴木　　猛
(JICA 北スマトラプロジェクト)

6 インドネシア・北スマトラのマラリア対策(2) 昆虫学的研究とベクターコントロール
鈴木　　猛，菊地　哲志
(JICA 北スマトラプロジェクト)
高木　正洋
(JICA 北スマトラプロジェクト，
予研・衛生昆虫)
安野　正之　　(公害研)

7 インドネシア共和国北スマトラ海岸地帯における塩水し好性蚊 *An. sundaicus* の棲息地近辺に住む人々の間の非常に高いマラリア伝播
糸川　英樹
(聖マリアンナ医大・病害動物)

8 *Trypanosoma burcei gambiense* は feedercell を選ぶか？
P. J. Mhando，柳　　哲雄，
中沢　秀介，福間　利英，神原　廣二
(長崎大・熱帯医研・原虫)

9 マウス J774･1 細胞を用いる *L. donovani* amastigote の *in vitro* cultivation: Carbocyclic inosine の amastigote に対する作用について
佐竹　弘靖，平岡　　修，綿矢　有佑
(岡山大・薬・薬品化学)
Jurieta Y. Kimura，森重　和久，
安治　敏樹，石井　　明
(岡山大・医・寄生虫)
松田　　彰，上田　　亨
(北大・薬・薬化学)

10 京都市における *Blastocystis hominis* の感染状況
山田　　稔，松本　芳嗣，手越　達也，
吉田　幸雄　　(京都府医大・医動物)

11 広範囲の大腸潰瘍および膿瘍を伴なった重症赤痢アメーバ症の1例
中村　敏夫　　(阪大・微研・原虫)
中村　　猛，網岡　勝見
(中村病院・枚方)

12 酵素抗体法による赤痢アメーバ症の診断（予報）
山浦　　常，白坂　龍曠
(東京女子医大・寄生虫)

13 東南アジアにおける疫病媒介蚊の羽音等を用いたトラップ法の応用
神田　錬蔵，小川　賢一
(聖マリアンナ医大・病害動物)

14 虫刺創の病理学的研究（第3報）
—免疫学的検討—
E. A. Jalal
(金沢医大・医動物，熱帯医研)
池田　照明　　(金沢医大・医動物)
佐藤　喜一，堀田　　進
(金沢医大・熱帯医研)

15 ヒトスジシマカ，ネッタイシマカのチクングニアウイルス感受性に及ぼす吸血の影響について
山西　　浩　　(神戸学院女子短大)
小西　英二，上野山登久
(神戸大・医・医動物)

16 沖縄本島における日本脳炎ウイルスの活動状況（1985年3月－1986年10月）
只野　昌之，石嶺　　毅，牧野　芳大，
安斎　俊一，福永　利彦

(琉球大・医・ウイルス)
長谷川英男　(琉球大・医・寄生虫)

17 デングウイルスの微量中和試験
石嶺　毅，只野　昌之，福永　利彦
(琉球大・医・ウイルス)
奥野　良信　(阪大・微研・防疫)

18 デングウイルスのエンベロープ糖蛋白質の解析
竹上　勉　(金沢医大・熱帯医研)
関　智代子　(金沢医大・血清学)
堀田　進　(金沢医大・熱帯医研)

19 モノクロナール抗体を用いたコレラの迅速診断実用化の研究
柳ヶ瀬康夫，青山　和枝，庄司　宏
(兵庫医大・細菌)
権平　文夫，松田　潤治，平野　勝，
杉山　純一，寺田　友次（デンカ生研)
C. P. Ranoa (San Lazaro 病院，マニラ)

20 バングラデシュで最近分離されたコレラ菌の生物学的諸性状
仲宗根　昇，岩永　正明
(琉球大・医・細菌)

21 バングラデシュおよび長崎で分離された *Aeromonas* 属についての比較検討
—薬剤感受性を中心に—
下口　和矩，餅田　親子，山口　恵三
(長崎大・病院・検査部)
草野　展周，内藤　達郎
(長崎大・熱帯医研・病原細菌)
河野　茂，斉藤　厚，原　耕平
(長崎大・医・二内科)

22 インドネシア各地の飲料水より検出された *Pseudomonas aeruginosa* の薬剤感受性と血清型について
奥脇　義行，矢内　寿恵
(女子栄養大・微生物)
藤田紘一郎，月館　説子
(長崎大・医・医動物)
杉山　雅俊　(順天堂大・医・衛生)

23 北アフリカ地域における飲料水の水質検査
澤田　清子，須永　寛　(瑞穂短大)

24 多孔質中空系と銀活性炭を組み合せた滅菌水装置の効力
小原　博，海老沢　功，高柳満喜子
(東邦大・医・公衆衛生)
小松　俊彦，甲原　照子
(予研・腸内ウイルス)

一　般　講　演 (25～68)
(次号掲載予定)

シンポジウム

Ⅱ　アジアにおける恙虫病

1　日本の恙虫病に関する医動物学的研究

浅沼　靖　(国立科学博物館・動物)

日本における恙虫病は，近年発生が著しく，1984年度には，患者数971名，発生都道府県数29に及んだ。この事実を「新潟，秋田，山形県下に限って発生する」とした過去の恙虫病に比べると今昔の感にたえない。

有毒地点と恙虫病の発生　ところで恙虫病発生地域の増加は，恙虫病リケッチア（以下Rと略）の大規模な分布拡大によるものではなく，元来存在した有毒地点の人における顕性化と考えているのであって，Tamiya (1962) は鼠類のR陽性地点を，すでに46都道府県に見出している。R感染鼠は有毒ツツガムシの存在を示すが，後者が全て恙虫病発生に連るとするには問題があり，北海道の如く，札幌，江別などにR感染例，カワムラツツガムシ（以下ツツガムシは略），ホッコクにR陽性例があるに拘らず，今なお患者未知の例がある。しかし後述のフトゲやタテの分布，特に前者の広いそれを考えると，一般には恙虫病発生の可能性を示唆するとしてもよいであろう。

新媒介者の証明　少なくともフトゲとタテの2種が媒介者となりうるとした所以は，浅沼ら (1962) にまとめたが，その後の追加事項としては，タテ—自然界での人寄生例（富士山麓，長崎），R陽性例（伊豆大島，富士山麓）。フトゲ—R陽性例（秋田，伊豆大島，富士山麓，長野，岐阜，大分など），R媒介例（秋田，新潟，山形）がある。しかしイカオタマ，ホッコク，ムロトなどにR陽性例のあることを思うと，新媒介者をフトゲとタテの2種に限定するには，まだ問題が残る（浅沼，1983）。

自然感染Rの鼠体内での運命　前述のフトゲのR媒介例（秋田）は，同県下の雄物川河川敷に健康ハツカネズミ116頭を設置した実験によるが，フトゲ単独種—満腹回収後，Gilliam型Rを分離—の寄生をうけたハタネズミが同型のRに感染したことを強調しておきたい。本実験でR感染の確かめられたハタネズミは，その後，飼育観察を続け，臓器Rは感染数カ月後には分離不能—R陰性となること，血清反応（補体結合反応）は1年後にも陽性成績を示すことなどが明らかとなった。

各地の恙虫病媒介者　八丈島，白浜（千葉），富士山麓（静岡）ではタテ，三浦・伊豆両半島南部，竹田（大分）ではフトゲがすでに媒介者として報告されている。しかし富士山麓では，タテ発生の全く終息している4月 (1982) に患者発生（従来は9－12月）があり，タテ以外にも媒介種の存在するであろうことが問題となっている。秋田，新潟県では近年春秋の患者が目立つが，浅沼らが早くから指摘しているように，同時期に多発するフトゲには常に有毒個体があり，本種を春秋期の恙虫病媒介者とすることには，最早異論はないであろう。特に秋田県下の古典的有毒地である十文字地域では，アカによる患者に加えて，フトゲ媒介性恙虫病の発生が確認されている（浅沼ら，1971）。以上によると，(1)媒介種は恙虫病発生地間で異なることがある，(2)複数種が同一地域で媒介に与りうるということになるが，(2)の例には，フトゲとタテの組合せ—伊豆大島の両種におけるR陽性結果による—が追加される可能性もある。

4　*Rickettsia tsutsugamushi* の細胞内増殖機構に関する電子顕微鏡学的研究

多村　憲　(新潟薬科大)

培養L細胞系を用いて *R. tsutsugamusi* (Rt) の宿主細胞への侵入，細胞内増殖，および細胞からの放出について，電子顕微鏡学的に詳細に検討し，以下の結果を得た。

宿主細胞への吸着と侵入：Rt の宿主細胞への侵入は細胞表面への接着から始まる。この接着は

原形質分離を起こしたRtや熱またはUV照射により不活性化したRtでも起こるが，Rtをトリプシンで処理すると起こらなくなる。次にRtの接着した部分の宿主細胞膜が少し凹み，同時にRtの側面に沿って偽足様の凸起ができる。Phagocytosisの開始である。この場合Rtは必ず形態的にintactなものに限られ，原形質分離を起こしたものや，熱またはUV照射により不活性化したRtは宿主細胞表面に接着はするが，決してPhagocytosisされない。こうしてRtは宿主細胞のPhagosome中に取り込まれる。この時2種類のPhagosomeが見られる。一つは空胞膜がRtをtightに包み，Rtと空胞膜との間隔が30 nm程度のものである。もう一つはRtと空胞膜の間隔が100 nm以上と巾広いものである。後者の場合，内部のRtは形態が変形しており，Rtがリソソームなどの影響により不活性化されて行く過程のものである。一方前者のtight phagosome中のRtは形態的にintactであり，正常な活性を保持していると思われる。このようなtight phagosomeには空胞膜の一部が断裂しているものが認められる。その断裂部分からRtは空胞中から脱出し，宿主細胞中に放出されるのである。すなわちRtの宿主細胞への侵入は，細胞表面への接着，Phagocytosisの誘発とPhagosome中への取り込み，Phagosomeからの脱出，という過程で進行する。

細胞質内増殖と細胞からの放出：Rtは長軸の中央部にくびれが生じ，2分裂により増殖する。Rtの数は経日的に増加するが，感染1日目では内部構造が比較的粗なRtが見られる。しかし旺盛な発育を示す感染3日目頃には電子密度の高い内部構造をもったRtが多数細胞質中に見られ，形も比較的均一な短桿菌状（0.5−0.7×1.0−2.0 μm）のものが大部分となる。しかし時に，異常に長いRt（0.7×5.0−8.0 μm）も認められる。また非常に稀ではあるが，宿主細胞核中で増殖するRtも見られる。感染3日目になると増殖した細胞質内Rtは細胞周辺部に移行し，細胞膜を内側から押し上げるように外に向かう。これは丁度enveloped virusが細胞表面からbuddingする形式に似ている。感染4日目になると細胞表面に多数のbudding Rtが蓄積し，殆ど細胞表面を埋めつくすほどになる。感染5日目になると細胞はcpeを起こして崩壊し，budding Rtおよび細胞内Rtは外液中に放出される。このようにRtの増殖サイクルは4－5日で，doubling timeは約9時間である。

一 般 講 演

1 マラリヤ原虫の迅速・簡易染色法——マラリアのスクリーニング診断およびマラリア原虫の薬剤感受性試験への応用

及川陽三郎 (金沢医大・医動物)
E. A. Jalal (金沢医大・熱帯医研)
谷 荘吉 (富士通・川崎病院)

マラリア患者の血液中原虫寄生率を求める場合，血液塗抹染色法は標本作製がやや煩雑であるうえ，鏡検に多くの時間を要する。演者らは，この操作を簡便化する目的で，*Plasmodium berghei* を用いてマラリア原虫の迅速・簡易染色法を考案し検討した。その結果，標本作製および鏡検に要する時間が大幅に短縮され，寄生率は塗抹染色法による結果とほぼ同等であることを第55回寄生虫学会で報告した。今回は，この染色法のマラリアのスクリーニング診断，およびマラリア原虫の in vitro での薬剤感受性試験における染色法への応用について検討した。

方法および結果：スクリーニング用には，ゲンチアナ紫原液を 0.3% EDTA–2Na·10% メタノール加生食水で50倍に稀釈して染色液とした。マラリア感染マウスの血液をピペットで直径約 3 mm になるように指頭上に置き，これをスライドグラスに触れさせて移し (約 0.008 m*l*)，そのうえに毛細管ピペットで本液を 1 滴たらし (約 0.025 m*l*)，混合後 24×50 mm のカバーグラスをかけて鏡検した。赤血球内原虫はほぼ球形で濃紫色に染色され，寄生率が 0.1%前後でも容易に原虫を検出することができた。薬剤感受性試験用にはゲンチアナ紫原液を 0.15% EDTA–2Na·10% メタノール加生食水で400倍に稀釈して用い，培養血液を本液で 5 倍に稀釈染色し血球計算盤で寄生率を求めた。キャンドルジャーでネズミマラリア感染赤血球を塩酸キニーネ添加培養液で 2 日間培養したところ，寄生率が対照と比較して有意に減少することが観察され，塗抹法による結果と一致していた。

考察：マラリアの診断において血液塗抹染色標本を用いることはマラリアの種類の鑑別には必要であるが，標本作製がやや煩雑であるばかりでなく，鏡検倍率1,000倍で油浸レンズを用いるため観察に多くの時間と熟練を要する。本法は，標本作成が容易でありまた鏡検も400倍で充分である。即ち，マラリア流行地においてマラリア患者のスクリーニングを現場で行う上で極めて有用であると思われる。

2 マラリヤ原虫 (*Plasmodium berghei*) 感染赤血球膜からの蛋白質分解酵素の抽出とその二，三の性質

井上 文英，徳 誠吉 (琉球大・医・一生化学)
松山 玲子 (琉球大・RI 実験施設)
佐藤 良也 (琉球大・医・寄生虫)

マラリア原虫 (*P. berghei*) に感染したマウス赤血球の形態は不規則に変化しており (Arnold *et al.*, 1969)，その赤血球膜ゴーストの SDS-PAGE では，赤血球膜の形態機能を保持しているスペクトリンとバンド 5 (アクチン) (Mueller and Morrison, 1981) が減少，または消失していることを認めた。一方，赤血球膜の機能維持に関与するエンドジニアスなプロティンキナーゼ (Shohet and Greenguist, 1978) 活性についても正常赤血球の酵素活性に比べて80%近くも減少していることを認めた (井上ら，1985)。

他方，マラリア原虫が宿主赤血球内で成長して行く過程で，宿主ヘモグロビン分子をアミノ酸源にしていることは知られている (Sherman, 1979) が，更に赤血球膜の構成蛋白質をも加水分解してアミノ酸源として利用していると考えられる。それで，マラリア原虫に感染すると，原虫の蛋白質分解酵素が赤血球膜の構成蛋白質を加水分解することにより赤血球の形態変化を導くと同時に，赤血球膜の機能低下を誘引していくと考えた。

そこで私共は，マラリア原虫に由来する蛋白質

分解酵素を得る目的で，エキソペプチダーゼの一種で原虫の代謝に必要なアミノペプチターゼを次の様な方法で抽出した。本酵素活性には合成基質の L-alanine-p-nitroanilide か L-leucine-p-nitroanilide を用いた。まず，マラリア原虫（*P. berghei*, NK-65 株）に感染した，BALB/C, ♀，8 週齢マウスの赤血球膜ゴーストを調製した。本酵素は，この膜ゴーストから非イオン性界面活性剤 Triton X-100 で可溶化（抽出）してセファデックス G-100 でゲルろ過を行い，次いで DEAE-セファデックスでイオン交換クロマトグラフを行って精製した。更に，この精製したアミノペプチダーゼについての物理化学的性質を検討した。

3 *Plasmodium berghei* 感染マウスにおける血液幹細胞の変動について

浅見　鳴子，丸山　治彦，大橋　　真，
名和　行文　　（宮崎医大・寄生虫）

マラリア感染において，宿主赤血球は原虫の侵入破壊を受ける。同時に感染赤血球の処理や原虫に対する免疫応答機序により，マクロファージやリンパ球を含めた白血球系細胞にも，大きな変動が見られる。ところが，これらの各種血球系の起源である血液幹細胞のマラリア感染時における動態については，殆ど知られていない。そこで今回私達はネズミマラリア（*P. berghei*）感染マウスを用いて感染に伴う骨髄幹細胞の変動を調べてみた。

8 週齢の C57BL/6 マウスに同系マウスより得た感染赤血球 10^5 個を i.p. 接種して感染させ，経時的に大腿骨より骨髄細胞を得た。骨髄細胞中の多分化能血液幹細胞数を定量する為に，5×10^5 個の細胞を致死量X線照射された同系マウスに静注し，一週間後に脾臓に形成さたコロニー（CFU-S）数を実体顕微鏡下で算定した。また CFU-S より一段分化の進んだ血液幹細胞の数を調べる為に，1×10^5 個の骨髄細胞をコロニー刺激因子（CSF）存在下で軟寒天培地中で一週間培養し，in vitro で形成されたコロニー（CFU-C）数を算定した。その結果，感染後 2 日目より CFU-S の上昇がみられ 8 日目のピーク時には，骨髄細胞 1×10^5 個あたりで感染前の約 2 倍となった。しかしながら14日目には，正常値以下となった。CFU-C は感染 2 ～ 4 日目にやや減少するが，6 ～10日目には感染前の 2 倍近くまで上昇し，14日目には正常値以下となった。^{59}Fe 静注により骨髄，および脾臓での鉄取り込みを調べてみると，4 日目より特に脾臓での有意な上昇が見られた。

これらの結果から，*P. berghei* 感染後早い時期から血液幹細胞が影響をうけて，感染の進行と共に大きく変動することが示された。

4 *Babesia* 感染に対するトキソプラズマ抗原（TLA）投与マウスにおける T-リンパ球の動態

鈴木　直義，関口　信一，佐藤　基佳，
桜井　治久，斉藤　篤志，広瀬　恒夫
（帯広大・獣医）
尾崎　文雄　　（神戸市）

TLA 乳剤投与マウスにおける *Babesia* 感染に対する抵抗性は，TLA 非投与マウスに比較して明らかに増強される。そこで，TLA 感作によるマウス体内のリンパ球，特に T-リンパ球および T-リンパ球 Phenotypes の動態について検討した。

トキソプラズマ破砕溶解抗原（144,000G，2 時間超遠心上清成分）100 μg と FIA の乳剤を14日おきに 2 回，筋肉内投与した。初回 TLA 投与から28日目に *B. rodhaini* 感染赤血球 1×10^5 個を腹腔内接種した。接種前 1 日目と接種後10日目の胸腺，脾臓，肝臓内 Thy-1（+）細胞（T-細胞）と SIg（+）細胞（B-細胞）数を計測した。*Babesia* 接種後10日目の胸線萎縮は両群とも著明であったが，TLA 感作によって胸線内 Thy-1（+）細胞数は，非感作マウスの約 2 倍以上に増数した。同様に脾臓内 Thy-1（+）細胞数も，*Babesia* 接種前 1 日で TLA 無処置マウスに比較して 3 ～ 6 倍に増数し，接種後10日では更に増加した。TLA 感作マウスの肝内 Thy-1（+）細胞は接種前 1 日で 0.3×10^7 個と計算されたが，接種後10日目で 3.4×10^7 個と肝内集積が最も著明であった。肝臓の病理組織学的および免疫組織学的検索では，RES の増殖と活性化および囲管性の単核球の著しい集簇が観察され，これらの単核球は主として，

Ly+^{1,2} (−), Ly+^{2,3} (+) の Thy-1 陽性細胞であった。

5 インドネシア・北スマトラのマラリア対策

(1) Primaquine 一回投与の *P. f.* gametocyte に対する効果

金子　明
(JICA 北スマトラプロジェクト, 弘前大・医・寄生虫)
亀井喜世子
(JICA 北スマトラプロジェクト, 帝京大・医・寄生虫)
石井　明　(岡山大・医・寄生虫)
鈴木　猛
(JICA 北スマトラプロジェクト)

マラリア流行地における，熱帯熱マラリア患者の chemotherapy では，schizontocide による trophozoites の clear のみならず，媒介蚊への感染形である gametocytes の消長に重大な関心が持たれる。当プロジェクトエリア内の Kuala Tanjung, Nana Siam の両村において，1985年 8 月から12月の間に，passive case detection を通じて見い出された熱帯熱マラリア患者の内，Fansidar 1,575 mg のみ一回投与群131例，Fansidar 1,575 mg＋Primaquine 45 mg の組み合わせ一回投与群87例について，投与後の gametocyte の消長を追跡調査した。尚，両群の全例において trophozoites は，Day 7 の時点で clear されており，その後 recrudescense は見られなかった。

Fansidar のみ投与した群では，Day 0 と Day 2 を対応できた25例，Day 0 と Day 7 を対応できた99例に，gametocyte の有意な変動は認められなかった。

Primaquine を組み合わせた群では，Day 0 と Day 2 を対応できた29例に gametocyte の変動は見られなかったが，Day 0 と Day 7 を対応できた72例では，Day 0 において gametocyte positivity rate (GR) 73.6%, gametocyte density (PDI) 2.2 であったのが Day 7 では GR 33.3%, PDI 1.3 となった。この減少は Wilcoxon's test により，1% 水準を持って有意であった。

更に Day 7 で gametocyte の残存していた例に対しては，それが消えるまで毎週再検をくり返した。Fansidar のみ投与群52例では，Day 0 での GR 71.1% が Week 2 まで維持され，その後下降して行ったが，Week 5 でも GR 11.5% が残った。Primaquine を組み合わせた56例では，Day 0 での GR 76.7% が day 7 ですでに30.3%と減少し，Week 2 で更に減り，Week 3 の時点で GR 7.1% となった。

今回の結果により，Primaquine 45 mg 一回投与を schizontocide と組み合わせる事が，*P. f.* gametocyte の消退を相当程度早めるのに効果があることが明らかになった。

6 インドネシア・北スマトラのマラリア対策

(2) 昆虫学的研究とベクターコントロール

鈴木　猛，菊地　哲志
(JICA 北スマトラプロジェクト)
高木　正洋
(JICA 北スマトラプロジェクト, 予研・衛生昆虫)
安野　正之　(公害研)

研究地域沿岸マラリアの媒介種は *An. sundaicus* で，当地のこの種は exophily のため，DDT 残留噴霧は効果を期待し得ない。そのため，integrated control による発生源対策を目的に，1978年以来基礎研究を続けてきた。また，1986年 9 月以降，32 km^2 の実験地域において，trial operation を実施中である。

対策としては，source reduction/environmental management を主要な柱とし，発生源の状況に応じてグッピーの放飼，殺虫剤（アベート）の散布を併用する。

海岸の砂丘後背地に散在するラグーンは，これを横に連ねるチャンネルを掘削し，水を近くの川に流すことによって，発生を封ずることが出来た。別の川の河口閉塞によって生じた氾濫原は面積が広く，魚や殺虫剤による防除がむつかしい。その draining について対策を模索中，*sundaicus* 幼虫が日照の悪い水域から発生しないことから，不用の魚池をヤシの葉でおおい，90%の防除効果を得た。

生物的防除法としては，グッピーをメダンから運び放飼した。池の環境によってその効果は異な

るが，一度の放飼で4カ月間幼虫の発生をみなかった例もある。殺虫剤については，アベートを2週間に一度水量の1 ppm 散布し，良好な防除効果を得た。

これらの経験をもとにして近く本格的な operation を実施する予定である。実施にあたっては，community の参加による住民の自主的な防除活動を将来の目途とし，実際的な control manual をつくることを目標にしている。

7 インドネシア共和国北スマトラ海岸地帯における塩水し好性蚊 *An. sundaicus* の棲息地近辺に住む人々の間の非常に高いマラリア伝播

糸川　英樹
（聖マリアンナ医大・病害動物）

インドネシア共和国北スマトラにおいて，JICA による医療協力プロジェクトの活動の一つとして，マラリア対策が行われている。海岸地帯に多くのマラリア流行巣が存在している事は前回報告したが，今回媒介蚊である，*An. sundaicus* の棲息地近辺に住む人々の間でマラリア伝播がいかに高いかを調べるために，全住民を対象に血液検査を施行し，malaria endemicity を明らかにした。またこの地での *An. sundaicus* の sporozoite rate を調べ，蚊についても検討した。その結果，幼少児では60%と高い原虫陽性率であり，そして年齢と共に陽性率は下がり，中年以降では最低値となり10～20%となった。

また，寄生虫濃度についても同様な傾向を示し，原虫濃度の高い者は幼少児のみに限られていた。熱帯熱マラリア原虫生殖母体に関する結果が，もっとも顕著な傾向を示していた。これらの事実は，住民にはかなりな程度でマラリアに対する免疫が成立している事を示唆している。また蚊においても，今回の調査によって初めてスポロゾイトがこの地の媒介蚊 *An. sundaicus* から検出され，その陽性率は0.6% (2/341) と unstable な vector である *An. sundaicus* にしては高い結果が得られ，蚊の方からもマラリア伝播の高いことが証明された。

8 *Trypanosoma brucei gambiense* は feeder-cell を選ぶか？

P. J. Mhando，柳　　哲雄，
中沢　秀介，福間　利英，神原　廣二
（長崎大・熱帯医研・原虫）

Trypanosoma brucei gambiense (Tg) を培養するときに，侍養細胞 (feeder-cell) として ICR マウス新生仔由来の細胞を用いると，分離して間もない脳細胞 (NMBC) と筋組織細胞 (NMMC) は Tg の増殖を助けるが，分離後40日を経過し樹立した脳細胞，筋組織細胞は侍養細胞としての能力を失う。新しく分離された細胞でも，皮膚細胞や腎細胞は，Tg の増殖を助けない。新しく分離されたトリパノソーマは，長期マウスで継代されたトリパノソーマより培養するのが困難であると報告されているが，NMBC および NMMC は Tg の分離された時期に関係なく侍養細胞として有効であり，又 *T. b. rhodesiense* や *T. b. brucei* にも有効であった。培養トリパノソーマは，10^7/ml のレベルに達し，形態上血流型と酷似しており，マウスに感染可能であった。Tg を侍養する細胞を培養皿の半面に，他の半面に侍養しない細胞を播き，その上で Tg を培養すると Tg は前者の側でのみ増殖した。増殖速度の速い細胞はトリパノソーマの侍養細胞として適しないという報告もあり，前述の各細胞にその増殖をおさえるに足る最少量のX線を照射し，侍養細胞として用いてみたが，いずれの細胞においても Tg の増殖を助けることに関して変化は見られなかった。Tg が増殖する系では，Tg は侍養細胞の直上あるいは間隙に入って細胞と密な接触を保ち，巣状になって増える。以上のことにより，侍養細胞から増殖因子が出されていない（あるとしても限極された近傍でのみ有効である）と考えられ，細胞と Tg の間に密な接触をもたらすことが Tg の増殖を推進するのに必要であると考えられる。

9　マウス J774・1 細胞を用いる *L. donovani* amastigote の *in vitro* cutivation: Carbocyclic inosine の amastigote に対する作用について

佐竹　弘靖，平岡　　修，綿矢　有佑
（岡山大・薬・薬品化学）
Julieta, Y. Kimura，森重　和久，
安治　敏樹，石井　　明
（岡山大・医・寄生虫）
松田　　彰，上田　　亨
（北大・薬・薬化学）

目的：寄生原虫の核酸代謝系は，宿主とは異なっており，プリンヌクレオチドの新生合成系が欠損し，且つ寄生原虫特有のサルベージ経路を有している。我々は，抗寄生原虫剤を開発するにあたり，このことに着目し，ヌクレオシド・アナログの抗原虫作用の検索を行った。その結果，*Leishmania tropica* および *Leishmania donovani* 原虫 (promastigote) に対し，イノシンアナログである carbocylic inosine (C-Ino) が有効であることを見出し報告した。今回我々は，マウスマクロファージ系 J774・1 細胞を用いて，*L. donovani* amastigote の *in vivo* like cultivation system を確立した。さらに，このシステムを用いて，宿主の形態である amastigote に対する C-Ino の効果を検討したので報告する。

方法：マウスマクロファージ系 J774・1 細胞を lipopolysaccharide (0.5 μg/ml, DIFCO, *E. coli* 0127: B8) を含む RPMI 1640 培地で 1 日培養した。この J774・1 細胞と同数の *L. donovani* promastigote を加え，さらに 1 日培養し，2 回洗浄した後，C-Ino を100%増殖阻害濃度である 7.5 μM 加え，C-Ino 存在下で 5 日間培養した。また C-Ino を前投与し，2 時間後に *L. donovani* を加えた場合の効果も調べた。*L. donovani* を加えてから一定時間ごとに染色 (Diff-Quik) し，100個の J774・1 細胞内の *L. donovani* 原虫を数え，感染率を求めた。

結果：C-Ino 存在下で培養している J774・1 細胞内への *L. donovani* 原虫の感染率は C-Ino 投与から経日的に減少し，C-Ino 投与後 5 日目で J774・1 細胞内に原虫は殆ど見られなくなった。これに対し対照群では 5 日目において約60%の J774・1 細胞に原虫が認められた。また，C-Ino を前投与した場合，感染率を50%に抑えることができた。これらの結果により，C-Ino は *L. donovani* に対して，その promastigote だけでなく amastigote に対しても殺原虫作用をもつことがわかった。

10　京都市における *Blastocystis hominis* の感染状況

山田　　稔，松本　芳嗣，手越　達也，
吉田　幸雄　　（京都府医大・医動物）

Blastocystis hominis Brumpt, 1912 は，世界中に分布し，長い間，非病原性のヒト腸管内寄生酵母と考えられてきたが，近年 Zierdt 一派の一連の研究により本微生物が酵母ではなく原虫の一種と考えられるようになった。そして最近，この微生物が下痢の原因となる可能性も指摘されている。しかしこの微生物の研究は非常に立ち遅れており，人体寄生の微生物であるにもかかわらず不明な点が多い。今回演者らは，1983年 6 月から1986年 7 月の間，京都市における *B. hominis* の感染状況を調べた。まず糞便検査を行った1,251人を 5 つの group に分けた。Group 1 (14人) は外国人旅行者で，彼らは殆ど消化器障害を訴えており，その多くは東南アジア，インド，アフリカを経て来日した者である。Group 2 (79人) は，消化器障害を訴えて当大学付属病院を訪れた者であり，Group 3 (1,000人) は何らかの疾患あるいはその疑いで当大学の検査室に持ち込まれた検体 (入院，外来) である。Group 4 (122人) は京都市内の 2 カ所の精神薄弱児(者) 施設の者で，Group 5 (36人) はそこに勤める職員である。検査はまず糞便のルゴール染色で検索し，ついで位相差顕微鏡ならびにギムザ染色により同定した。その結果，Group 1 では14人中11人 (78.6%)，Group 2 では79人中 3 人 (3.8%)，Group 3 では1,000人中12人 (1.2%)，Group 4 では122人中21人 (17.2%)，Group 5 では36人中 1 人 (2.8%) に *B. homonis* を見出した。下痢を認めた *B. hominis* 陽性患者の中には，ランブル鞭毛虫，鞭虫など他の寄生虫

の感染も認められた。また *B. hominis* は便性状が有形ないし軟便でも認められた。*B. hominis* はわが国においても潜在的にヒトに感染しており，糞便を介して感染が広がるものと思われる。最後に協力を頂いた本学・臨床検査部の方々に深謝する。

11 広範囲の大腸潰瘍および潰瘍を伴った重症赤痢アメーバ症の1例

中林　敏夫　　(阪大・微研・原虫)
中村　　猛，網岡　勝見
(中村病院・枚方)

患者は48歳，男性，大阪市在住。約20年間，精神分裂症のため某病院に入院，加療中であった。海外渡航歴はない。1986年2月10日，頭部外傷で中村病院に入院，10日後に治癒，退院した。当時，右腹部の腫瘍の疑が持たれたが，対症治療を与えるに止まった。

6月22日，腹部膨満，便秘，発熱を訴え再入院した。イレウス病状が強く，腹壁瘢痕性ヘルニアを認めた。ロマノスコピーで腸粘膜に異常を認めなかった。X線像で大腸部にガス貯留が著明で，通過障害が認められた。保存的療法を試みたが効なく，6月27日に開腹手術を施行した。上行結腸に狭窄および壊死があり，盲腸は後膜壁と，右結腸曲部は肝および胆嚢と癒着していた。上行結腸後面に膿瘍形成が認められた。回腸末端部より横行結腸中間部までを切除し，腸吻合を行った。術後も病状は好転せず，意識レベルも次第に低下した。7月4日，切除結腸組織標本中に多数の赤痢アメーバ栄養型を検出した。

7月5日よりデヒドロエメチン (40 mg, 筋注，1日1回7日間)，メトロニダゾール (500 mg/日，胃内注入，7日間) 投与を開始したが，容態は悪化し7月14日死の転帰をとった。切除結腸粘膜は壊死におちいり，黄白色の厚い偽膜で被われていた。潰瘍は外膜に達し，穿孔寸前であった。潰瘍底部には多数の赤痢アメーバと共に好中球，単球等が認められた。腸組織にはリンパ球，形質細胞，単球等の他に繊維性増殖，血管の新生があり，壊死性肉芽性炎症像を呈した。本症は国内感染例で，長期間の経過の後に開腹手術後はじめて赤痢アメーバ症を診断されたものである。

12 酵素抗体法による赤痢アメーバ症の診断 (予報)

山浦　　常，白坂　龍曠
(東京女子医大・寄生虫)

近年，注目されている酵素抗体法 (ELISA) の赤痢アメーバ症への診断の応用性について検討した。抗原には HJ 株を使用し，標識抗体にはペルオキシターゼ標識抗 IgG 羊血清を，基質は，2,2′-azinobis (3-ethylbenzthiazoline sulfonic acid) を使用した。ELISA の手技は，Matsuda *et al.* (1984) に準じ，反応後のプレート (Dynatech, イミュロン1) の吸光度を直読式分光光度計 (MIP-32, コロナ) により 415 nm の波長で測定した。(1) 4°C, 23°C, 37°C の温度で抗原のプレートへの感作条件を検討した所，4°C 1日感作で高い吸光度が得られた。(2) 至適抗原濃度と標識抗体濃度の検討では，蛋白質として 10 μg/ml が抗原の至適濃度で，一患者一穴法 (血清希釈200倍) を利用するための標識抗体濃度は6,000倍が適した濃度と判定された。(3) ELISA (一患者一穴法) による赤痢アメーバ症の診断：一般健康人血清 (アメーバシスト陰性者) 200例について ELISA を実施した所全例が 0.200以下 (0.028～0.181) の吸光度を示したので0.200以上を陽性と判定した。その結果，アメーバ赤痢患者2例は，0.586，0.610 (平均 0.598)，アメーバ性肝膿瘍患者18例は，0.410～1.820 (平均1.020)，アメーバ性肝膿瘍と潰瘍性大腸炎の合併患者3例では，0.964～1.500 (平均 1.268) の吸光度を示し全例陽性と判定され，赤痢アメーバシスト陽性者血清32例では2例 (6.3%) のみが陽性であった。非アメーバ性肝膿瘍や大腸炎の患者血清6例は全例陰性であった。アメーバ症患者血清の ELISA 抗体価は，400倍が6例 (26.1%)，800倍が3例 (13.0%)，1,600倍が10例 (43.5%)，3,200倍以上が4例 (17.4%) で23例中6例 (26.1%) の患者は国内感染であった。詳細は現在検討中であるが，ELISA は赤痢アメーバ症の免疫学的診断に応用しうるものと思われた。

13 東南アジアにおける疾病媒介蚊の羽音等を用いたトラップ法の応用

神田　錬蔵，小川　賢一
（聖マリアンナ医大・病害動物）

疾病媒介蚊駆除法に殺虫剤を用いないで，蚊のもつ物理的生物的性質を逆手に，羽音吸血誘引性を利用してトラップする事の考案応用を試みた。これは，蚊のもつ羽音を記録し，これに合わせた振動数の音をスピーカーで発信し，一方これに金網ケージに実験動物を入れ，ドライアイスを用い，吸血誘引し，蚊を集める方法である。*Mansonia*, *Culex* および *Aedes* には，池庄司が考案した四角のプラスチック薄板を，スピーカーを中央に円筒形に巻き，粘着剤を噴霧し，飛来した蚊をトラップするものである。このスピーカーの下に動物ケージとドライアイス 0.5 kg を置いた。ハマダラカには円筒に代わり，小動物のハムスターを入れた小型ケージとスピーカーを直径約 30 cm のちょうちんに入れ，外側に粘着剤を噴霧した薄い塩化ビニール袋をかぶせ，ちょうちんと袋は予め多数の穴を開けて，音と匂いの発散を計った。羽音には誘殺目的の蚊と同じ振動数のものを用いた。こうすることにより，羽音だけで誘殺するよりも，動物とドライアイスを併用することでより多く蚊がトラップされ，ヌマカ類では 330 Hz から 350 Hz の振動数の羽音に最も多かった。マレーシアセランゴール州，ベルジュンタイ村の亜鉛採掘廃鉱に溜まった沼地の周囲の草叢において，同一ステーション連続 3 日毎 3 回の採集により，初回の870頭から 3 回目には 4 頭に減少し他の場所でも同様の傾向を示した。尚，間隔は 50 m を中心にした。一方タイ国アユタヤ省 Bang Pa Inn 部落の水田地帯で，1986年 5 月から10月にいたる月毎の消長をこの方法で調べたところ，8 月が最高で 4,590頭をトラップした。この前後の月は明らかに少なかった。各ステーションを比べ，密度の高い所は他よりも比較的高密度を示した。場所により密度の差をみたが，雄蚊はその発生源からの移動はないようである。*Culex tritaeniorhynchus* は吸血誘引による雌蚊のトラップが著明であったが，ヌマカではこれが特に多いとはかぎらなかった。ハマダラカの採集では，450 Hz から 530 Hz 間の振動数に雌雄共にトラップされている。

以上の実験から，羽音は同一種の雌の発する振動数を中心に，吸血誘引物質を併用することにより，ヌマカは雄が非常に多く誘殺され，イエカ属での吸血誘引物質の併用は，コガタアカイエカの雌を多く誘殺できた。ハマダラカ属は球形のトラップの方が効率が高かった。本法により個体群動態を推定することも出来，ヌマカ属の駆除に応用できるものと考えられる。

14 虫刺創の病理学的研究（第 3 報）
—免疫学的検討—

E. A. Jalal
（金沢医大・医動物，熱帯医研）
池田　照明　（金沢医大・医動物）
佐藤　喜一，堀田　進
（金沢医大・熱帯医研）

我々は，蚊刺咬暴露されたモルモットについて，腹腔細胞における肥満細胞脱顆粒反応および homocytotropic antibody response について検討し，さらに血清による感作状態の passive transfer についても検討を加えたので報告する。

材料および方法：雄 Hartley 系 albino モルモットの背部を週 2 回ずつ継続的に *Aedes ageypti* で蚊暴露し感作した。肥満細胞脱顆粒反応は，腹腔侵出細胞を蚊抗原加培養液で 37°C, 10分間 incubate して判定した。Homologous PCA 反応は24時間と 8 日間の latent period で行い，ELISA は抗原感作した96穴 microplete で peroxidase 標識 anti-モルモット IgG を用いて行った。蚊抗原は，蚊の頭部・胸部のホモジネートを PBS (pH 7.2) で抽出することにより得た。

結果：蚊暴露開始後 2 週目に発赤腫脹が出現し 8 週目まで増大したがその後縮小傾向がみられた。肥満細胞脱顆粒率は蚊暴露開始後 3 週目より上昇し，以後も高率に推移した。感作モルモット血清の24時間 PCA 反応では，7 週目から 9 週目をピークとする一過性の反応を示した。8 日間 PCA では，8 週目に高い抗体価が得られたのみだった。一方，56°C, 30分間の血清の熱処理により，24時間・8 日間 PCA とも抗体価の大幅な低

下が認められた。ELISA により測定した specific IgG 抗体は，5週目より抗体値が上昇し8週目にピークを示したが，その値は，極めて低く，以後抗体値は漸次減少する傾向が認められた。感作モルモットより得た IgE 抗体陽性血清の 1 m*l* を正常モルモットに静注し，8日目に蚊暴露して，発赤の出現を検討したところ，発赤が出現し，大きさも感作モルモットが示した最大平均値に匹敵するものだった。56°C, 30分間の熱処理により血清の感作能の低下が認められた。以上の結果より，蚊刺咬の結果できる発赤腫脹は，即時性アレルギー反応に起因するものであると結論された。

15 ヒトスジシマカ，ネッタイシマカのチクングニアウイルス感受性に及ぼす吸血の影響について

山西　浩　（神戸学院女子短大）
小西　英二，上野山登久
（神戸大・医・医動物）

ネッタイシマカ，2系統のヒトスジシマカおよびコガタアカイエカに，チクングニアウイルスを経口感染させ，中腸におけるウイルスの消長を調べた。感染直後，感染1日後には中腸のウイルス量は殆ど同じであった。感染2日後にコガタアカイエカではウイルスが全く検出されず，ネッタイシマカでも一部に少量検出されたにすぎない。ヒトスジシマカ兵庫系では，ウイルス量は少なかった。一方オアフ系では，ウイルス量がやや減少する程度であった。感染3日後ではオアフ系の中腸で 10^3 PFU 程度のウイルスが検出され，4日後には中腸以外の組織からもウイルスが検出された。感染7日後には兵庫系は中腸だけでウイルスがみられ，オアフ系では頭部，胸部で 10^6 PFU 以上のウイルスが検出された。14日後にはその傾向が顕著になった。このように蚊のウイルス感受性は，中腸におけるウイルスの消長に依存していた。ネッタイシマカにおいて，感染前の吸血あるいは再点検によって中腸でのウイルスの増殖への影響を調べた。今回の実験では，ウイルス増殖に及ぼす影響は，はっきり認められなかった。

16 沖縄本島における日本脳炎ウイルスの活動状況（1985年3月－1986年10月）

只野　昌之，石嶺　毅，牧野　芳大，
安斎　俊一，福永　利彦
（琉球大・医・ウイルス）
長谷川英男　（琉球大・医・寄生虫）

沖縄本島における日本脳炎ウイルス（JEV）の活動状況を知るために，沖縄本島で飼育された豚の JEV 抗体陽性率の調査，およびコガタアカイエ蚊（C.T.）の採集と採集された C.T. から JEV の分離を行った。

1985年3月から1986年10月までの調査期間で80%以上の抗体陽性率が見られた期間は，沖縄本島の北部では，85年6月から12月までの間と86年7月から10月までの間に見られた。中部地区は，85年7月下旬から10月下旬までの間と86年5月下旬から10月までの間に見られた。南部地区は85年8月中旬から9月下旬までの間と86年8月下旬から9月上旬までの間に見られた。調査期間を通しての陽性率は北部が50%，中部が47%，南部が30%で，北部が最も高く，南部が最も低い値を示した。3地区に共通して陽性率のはげしい変動がみられた。北部・中部・南部の3地区の抗体保有率の推移を合せてみると，抗体保有率が0%になることは殆どなかった（調査期間を通して抗体保有率が0%になったのは，86年6月11日の検体だけであった）。

2ME 感受性抗体は本島全体でみると，85年4月から10月の間と86年5月から10月までの間で検出された。

中部地区の3カ所の豚舎で，85年3月から12月までの間で採集された C.T. の数（1,233匹）に比べて，南部地区の一豚舎では86年5月から10月までの間に多数（10,875匹）の C.T. が採集された。中部地区のプールから1株，南部地区のプールから18株の JEV が分離された。南部地区の採集地点は，クレソンを栽培する水田に隣接しており，それが蚊の発生源と考えられる。中部地区の採集地点の周囲には発生源が見られなかったので，発生源の有無が C.T. の採集数の差に現われたものと考えられる。沖縄本島では水稲の作付が減少し

ており，「い草」「水芋」「クレソン」等の作付面積も発生源を考える上で重要であると考えられる。沖縄の北部，中部で高い抗体陽性率が長期間続いたこと，2ME 感受性抗体が6カ月間にわたって見られたことから，沖縄本島では1年間の内かなり長期間にわたって JEV が活動していることが強く示唆される。

蚊の発生源である水田が減少していることと，最近の豚の多頭飼育化の傾向とにより JEV の循環が起こりうる豚舎が孤立化しているのではないかと思われる。そのために個々の豚舎で独立して JEV が循環し，豚の JEV に対する抗体陽性率の推移が大きく変動したものと推察する。

17 デングウイルスの微量中和試験

石嶺　毅，只野　昌之，福永　利彦
（琉球大・医・ウイルス）
奥野　良信　（阪大・微研・防疫）

東南アジアのある地域では，フラビウイルスに属するデング (DEN) ウイルス，日本脳炎 (JE) ウイルスが共存している。DEN ウイルス 1–4 型のサブグループ，JE ウイルスの間では赤血球凝集抑制 (HI) 試験で交差反応がみられ，HI 試験でのウイルスの区別は難しい。血清学的に最も特異性の高い方法は中和 (N) 試験であるが，従来のプラーク法では接種後の incubation period が長く，使用する検体血清量も多い。また，多数の検体を処理する血清疫学的研究には大量の器材，労力等が要求される。8チャンバースライドとペルオキシダーゼ—抗ペルオキシダーゼ (PAP) 染色法を併用した迅速な DEN, JE ウイルスの中和法が奥野ら (1978) によって開発されたが，8チャンバースライドが高価で，多数の検体を扱う場合に問題がある。今回，8チャンバースライドの代わりに96穴プレートを用い，DEN ウイルスの中和を試みた。96穴プレートではウイルス希釈とフォーカス数は良く相関し，DEN2，4型では接種後40時間，DEN1，3型では56時間後に計数しやすいフォーカスサイズを示した。N試験では8チャンバースライド，96穴プレートで得られたN抗体価は2倍以内の範囲にあり，良く相関した。96穴プレートを用いたN法は8チャンバースライドに比べて，安価であり，多数の検体を少量で迅速に処理できるので，DEN ウイルスの大規模な血清疫学的研究に広く応用できる。

18 デングウイルスのエンベロープ糖蛋白質の解析

竹上　勉　（金沢医大・熱帯医研）
関　智代子　（金沢医大・血清学）
堀田　進　（金沢医大・熱帯医研）

デングウイルス感染成立の過程においてエンベロープ (E) 糖蛋白質は重要な役割を果たすと考えられているが，生化学的，免疫学的解析は十分とは言えない。本研究は，E蛋白質に対する単クローン抗体の調整と抗原分析，並びにE蛋白質領域の遺伝子構造の解明をめざし解析を行った。

マウス Balb/c にデングウイルス1型望月株を接種し，追加免疫後4日目に免疫マウス脾細胞を採り，更に脾細胞と SP 2/0 ミエローマ細胞とを融合させ，ハイブリドーマを得た。デングウイルス粒子を抗原に用いた ELISA 法による2回のスクリーニングの結果，20種以上の単クローン抗体を得た。ELISA 価および血球凝集阻止 (HI) 抗体価の調査から，得られた単クローン抗体は少なくとも3種のグループに分けることができた。即ち，(1)ELISA 価，HI 価が共に高い，(2)ELISA 価は高いが HI 価は低い，および(3)ELISA 価は低いが HI 価は高い等の各グループになった。いずれのグループに属する抗体も免疫沈降法によってデングウイルスE蛋白質と特異的に反応することを確認した。他方，エンベロープとの関わりが予想されるE蛋白質のC′末端側を調査する目的で逆転写酵素および合成プライマーを利用するヌクレオチドシークエンシングを行った。E蛋白質のC′末端側は，非電荷アミノ酸が20個以上連続して配列している疏水性領域であることを明らかにした。又，E蛋白質と隣接する蛋白質との切断部位は，黄熱ウイルス等と同じくアラニンーアスパラギン酸であった。以上の事実からE蛋白質のC′末端領域はエンベープに挿入しており，その他の領域は外界と接する部分であり，HA 部位を含めいくつかの抗原決定部位が存在することを示唆している。

19 モノクロナール抗体を用いたコレラの迅速診断実用化の研究

柳ヶ瀬康夫，青山　和枝，庄司　宏
（兵庫医大・細菌）
権平　文夫，松田　潤治，平野　勝，
杉山　純一，寺田　友次（デンカ生研）
C. P. Rañoa（San Lazaro 病院，マニラ）

従来，コレラ菌の検出同定は，患者検体より分離培養後に生化学的，血清学的同定を行うが，コレラの特性上迅速かつ確実な診断が必要である。我々は患者検体よりコレラ菌の直接検出を目的としてホルマリン不活性化菌を免疫原とし，コレラ菌および小川型，稲葉型に特異的なマウス腹水型モノクローナル抗体を作製した。この抗体を hydroxylaptite gel によるカラムクロマトグラフィーで精製した。精製抗体をラテックス粒子に感作して，各クローンの抗体感作ラテックスをスライド凝集反応用試薬として検討を加えた。

使用したA感作ラテックスは小川型，稲葉型標準株を特異的速やかに凝集して，O1 抗原因子Aを認識し，B感作ラテックスは小川型のみに，C感作ラテックスは稲葉型のみに凝集を示し，それぞれ抗原因子B，Cを認識したクローンであると考えられる。

そこでフィリピン国立サンラザロ伝染病院において，このA感作ラテックス試薬を用い下痢患者検体より直接検出と TCBS 平板，TSI による培養試験を平行して行い，モノクローナル抗体感作ラテックスによるコレラの迅速診断実用化の検討を加えた。

1985年，54検体中本試験薬による直接検出および TCBS 培養試験陽性になったものが18例，直接検出陰性で培養試験陽性になったものはなかった。

1986年，検体をアルカリ性ペプトン水に1/100接種，2時間増菌培養したものについて検討した。254検体中，①直接検出陽性，培養試験陽性39例。②直接検出陰性，培養試験陽性17例，これは定量培養で $10^{2\sim4}$/ml 以下で本試薬の検出感度に達しなかった為である。③直接検出陽性，培養試験陰性3例，これは 100°C，10分間加熱処理で凝集陰性となった。

以上本試験は患者検体からのスクリーニングに優れた特異性，検出感度を示し，コレラの迅速診断に有用と考えられる。

20 バングラデシュで最近分離されたコレラ菌の生物学的諸性状

仲宗根　昇，岩永　正明
（琉球大・医・細菌）

1986年1月コレラ患者から分離されたコレラ菌91株の生物学的性状，および6種の抗菌剤に対する感受性について調べた。血清型において，79株が小川型，10株が稲葉型，2株が彦島型であった。コレラファージⅣとポリミキシンBに対する感受性により，91株中60株が classic 型，31株が El Tor 型に分類され，1973年以来姿を消し，1982年再び突如として現れた classic 型コレラ菌は，現在もなおバングラデシュにおいて優勢であった。El Tor 菌31株の溶血性で，血液寒天平板上で β-溶血を示したのは21株 (67.7%)，Feely and Pittman の方法で完全溶血を示したのは8株 (25.8%) であった。ファージタイプで，El Tor 菌は Type 1, 4, 5, untypable に分れたが，classic 型菌は全株，Mukerjee の Type I に属した。El Tor 型菌のプロファージ型は，ウボール型14株，セレベス cured 16株，セレベス原型1株であり，ウボール型が多いのが特徴的であった。毒素産生を培地または培養条件を変えて調べてみると，当教室で考案した El Tor 型毒素産生用培地の AKI 培地を用いると，今回分離された classic 株も高い毒素産生を示し，1973年以前に分離された classic 型コレラ菌と相違を示した。6種の抗菌剤に対する MIC は，多くの株がクロラムフェニコールで 0.78 μg/ml，ストレプトマイシン，アモキシシリンで 6.25 μg/ml を示し，ミノマイシン，テトラサイクリン，ナリジクス酸に対しては 0.2 μg/ml 以下と，6薬剤に対し高度耐性株はみられず，1980年頃，バングラデシュにおいて問題となったテトラサイクリン耐性コレラ菌は消失していた。

21 バングラデシュおよび長崎で分離された *Aeromonas* 属についての比較検討 —薬剤感受性を中心に—

下口　和矩，餅田　親子，山口　恵三
（長崎大・病院・検査部）
草野　展周，内藤　達郎
（長崎大・熱帯医研・病原細菌）
河野　　茂，斉藤　　厚，原　　耕平
（長崎大・医・二内科）

目的：今回私達は，バングラデシュの国際腸管感染症センター（ICDDR. B）より分与された *Aeromonas* 属の薬剤感受性について検討を加え，長崎にて分離された臨床分解株の成績と比較したので報告する。

対象および方法：供試菌株としては，ICDDR. B から分与された人，環境由来の105株（*A. hydrophila* 29株，*A. caviae* 41株，*A. sobria* 35株）と長崎にて分離された臨床分離株29株（*A. hydrophila* 14株，*A. caviae* 12株，*A. sobria* 3株）の計134株を用いた。

薬剤感受性試験は，16薬剤（ABPC, MPC, PIPC, CEZ, CEX, CCL, CTM, CZX, CP, GM, KM, TOB, TC, MINO, ST, NFLX）を用いて，ミクロブロス希釈法（MIC, 2000, Dynatech）による12濃度法にて行った。

成績：ICDDR. B 分与株と長崎臨床分離株を対象とした上記16薬剤の薬剤感受性試験の成績には，著しい相違点は認められず，両者とも ABPC, CEZ, CEX, CCL には耐性を示す株が多く存在したが，その他の薬剤に対してはほぼ全株が感性を示した。一方，菌種別に薬剤感受性成績を比較してみたところ，*A. hydrophila, A. caviae* では殆どの株が ABPC, CEX, CEZ, CCL に耐性を示したが，*A. sobria* は ABPC に対して約50%の株が感性を示し，また，CEX, CEZ, CCL に対してはそれぞれ約10%，30%，30%の株が感性を示した。次に，菌株毎の薬剤耐性パターンについて比較してみると，*A. hydrophila, A. caviae* では，ABPC, CEX, CEZ などに多剤耐性を示す株が殆どであったが，*A. sobria* は CEX のみに耐性を示す株が約半数に認められた。これら耐性機序の一つに，β-ラクタマーゼ産生性の関与が考えられたため，ABPC, CEX に耐性を示す *A. hydrophila* を対象に，ミクロヨード法にて，そのβ-ラクマターゼ基質特異性を検討したところ，本菌はペニシリナーゼ産生菌であることが示唆された。

22 インドネシア各地の飲料水より検出された *Pseudomonas aeruginosa* の薬剤感受性と血清型について

奥脇　義行，矢内　寿恵
（女子栄養大・微生物）
藤田紘一郎，月舘　説子
（長崎大・医・医動物）
杉山　雅俊　　（順天堂大・医・衛生）

今回は，1985年および1986年に行った，インドネシア各地の飲料水の細菌学的調査で検出された *Pseudomonas aeruginosa* の化学療法剤感受性テスト，および血清型別の成績を報告する。

Pseudomonas aeruginosa は，1985年は70検水中から11菌株，1986年は98検水中から19菌株分離された。

これらの菌株について，PCB, PIPC, MZPC, CER, CET, CFX, CMZ, CPZ, GM, KM, CP, TC, CL および NA の14剤の感受性テストを，まずディスク法で行った。その結果，PCB, PIPC, MZPC, GM, KM, CP, TC, CL および第3世代セフェム剤の CPZ には良好な感受性が示された。しかし，第1世代セフェム剤の CER, CET, 第2世代セフェム剤の CFX, CMZ には耐性であった。

そこで，1986年に分離された19菌株について，ディスク法で耐性を示した薬剤の MIC 値を求めた。その成績は，19菌株の殆どで CER 1,000 μg/ml 以上，CET 250 μg/ml 以上，CFX 1,000 μg/ml 以上，CMZ 500 μg/ml，および NA 1,000 μg/ml という極めて高い値を示した。

一方，疫学的見地から重要視される *Pseudomonas aeruginosa* の血清型を調べたところ，1985年の11菌株では6株がE型であった。また1986年の19菌株では，E型とF型がそれぞれ6株ずつであった。

23 北アフリカ地域における飲料水の水質検査

澤田 清子，須永 寛 (瑞穂短大)

1984年8月，モンロビア，アビジャン，コナクリ，アルジェ，チュニスおよびトリポリの6都市において，水道水，レストランや邦人宅の飲料水並びに市販の容器入り飲料水について，水質検査を行って次の成績を得た。

水道水でも遊離残留塩素が検出されないものが2/3に達し，殊に大西洋沿岸の3都市とトリポリに多かった。また，これらの地域の水道水から大腸菌群が分離された。

モンロビアにおける食堂や，ホテルの飲料水からは高率に大腸菌群が検出された。在留邦人家庭の飲料水からも，大腸菌群が検出される例があった。これらの飲料水は，いずれも濾過器を通したものであり，その使用法や管理が適切でないためと考えられた。一方，市販の容器入り飲料水からは，大腸菌群は検出されなかった。

飲料水80検体のうち，大腸菌群が陽性のものは20検体であった。また，分離された21株を菌種別にみると，*K. pneumoniae* が1/3で最も多く，*Ent. cloacae, C. freundii, K. oxytoca* の順であり，*E. coli* も2株検出された。種々の薬剤に対する感受性検査 (MIC) では SM, TC, CP に感受性の低いものが少数例みられたが，ピペミッド酸にはすべてが高い感受性を示した。

腸菌球の陽性は6検体で，7株が分離された。そのうち5株は *St. faecalis liquefaciens* で，*St. faecalis* と *St. faecium* が1株ずつであった。なお，腸球菌陽性の検体はいずれも大腸菌群も陽性であった。

水道水の化学的検査では，過マンガン酸カリウム消費量が 10 mg/*l* 以上のものが過半数を占めていた。総硬度および塩素イオン濃度は，アルジェおよびトリポリの水道水の殆どが，飲料水の水質基準値を越えていた。殊にトリポリでは，塩素イオン濃度が 1,000 mg/*l* 以上のものもみられ，塩辛い水は日常生活に支障を来していた。

24 多孔質中空系と銀活性炭を組み合わせた滅菌水装置の効力

小原 博，海老沢 功，高柳満喜子
(東邦大・医・公衆衛生)
小松 俊彦，甲原 照子
(予研・腸内ウイルス)

水より感染する疾患は，開発途上国では極めて高頻度である。海外に滞在する日本人が増加するに伴い，水の衛生がますます重要な課題となっており，その有効な対策が望まれている。近年，多孔質中空系と銀活性炭を組み合わせた滅菌水装置(三菱レイヨン・日本ケミファ) が開発され期待が持たれている。その効力を調べるため，特に開発途上国滞在中に水より感染し易いと思われる病原体を用いてその滅菌性能について実験を行い，検討した。

実験菌としては，大腸菌，セラチア，腸チフス菌，コレラ菌，赤痢菌，ネズミチフス菌を用いた。10^8～10^9 個の生菌を含んだ蒸留水 1*l* を滅菌水装置に通し，得られた液をミリポアフィルターに通した。フィルター上部を遠藤培地に接種して一晩培養し，集落数を測定した。滅菌水装置に菌液を直接通す実験の他に，水道水 50*l* または多摩川の水 15*l* を通す前処置を施した後，1週間放置して菌液を通す実験も行った。菌液中の菌数は普通寒天培地を用い，段階希釈法で測定した。ウイルスについては，ロタウイルスを用いて検討した。

6種類の細菌について行った実験の結果，滅菌水装置を通した後の液中には菌はまったく検出されなかった。水道水，または多摩川の水を通す前処置を施した後でも，液中に菌はまったく検出されなかった。ロタウイルスについて行った実験の結果もウイルスは検出されなかった (堀田ら，1984)。

主要な腸内細菌およびロタウイルスについて行った実験の結果，病原体は検出されず，良好な性能を有すると思われた。多孔質中空系と銀活性炭を組み合わせることにより，細菌はもちろんウイルスの除去にもかなり効果が期待できる。飲食物に関する注意や予防接種に滅菌水装置を加えることにより，経口感染症の予防に大いに役立つと思われる。

Japan. J. Trop. Med. Hyg., Vol. 15, No. 2, 1987, pp. 139–168

PROCEEDINGS OF XXVIII ANNUAL MEETING OF JAPANESE SOCIETY OF TROPICAL MEDICINE (1)

22–23 November 1986 Okinawa

CONTENTS

7 Extremely high transmission of malaria among those who live near breeding sites of the sea-water mosquito, *An. sundaicus*, in the coastal area of north Sumatra, Indonesia H. Itokawa

8 Does *Trypanosoma brucei gambiense* choose feeder-cells? P. J. Mhando *et al.*

9 Anti-parasite activity of carbocyclic inosine against amastigotes of *Leishmania donovani* H. Satake *et al.*

10 The prevalence of *Blastocystis hominis* infection in humans in Kyoto city M. Yamada *et al.*

11 A serious case of amebiasis accompanied by extensive colic ulcer and abscess T. Nakabayashi *et al.*

12 Serological diagnosis of amoebiasis by micro-ELISA H. Yamaura and R. Shirasaka

13 Use of wing sound traps for male mosquitoes of some vector species in fields T. Kanda and K. Ogawa

14 Pathological study on mosquito-bitten wound (III) An immunological study E. A. Jalal *et al.*

15 The influence of blood sucking on the susceptibility of *Aedes albopictus* and *Aedes aegypti* to CHIK virus H. Yamanishi *et al.*

16 The state of Japanese encephalitis virus activities in Okinawa main island M. Tadano *et al.*

17 Micro-neutralization test of dengue viruses using 96-well plate and peroxidaseanti-peroxidase technique T. Ishimine *et al.*

18 Analysis of the envelope glycoprotein of dengue virus T. Takegami *et al.*

19 Monoclonal antibody-based reversed passive latex agglutination for identification of *Vibrio cholerae* O1 Y. Yanagase *et al.*

20 Characterization of *Vibrio cholerae* O1 recently isolated in Bangladesh N. Nakasone *et al.*

21 Comparative study on drug susceptibility of *Aeromonas* species isolated in Bangladesh and Nagasaki K. Shimoguchi *et al.*

22 Drug sensitivity and serotype of *Pseudomonas aeruginosa* isolated from drinking water in Indonesia Y. Okuwaki *et al.*

23 Examination of drinking water in northern African countries K. Sawada and H. Sunaga

24 Water-sterilizing capacity of an equipment made of hollow multi-porous fibers and silber-activated carbon powders H. Ohara *et al.*

25–68 will be appeared in the next issue

Spesial lecture

PUBLIC HEALTH ASPECTS OF TROPICAL DISEASES IN THE WESTERN PACIFIC REGION

HIROSHI NAKAJIMA
Regional Director, WHO/WPRO, Manila, Philippines

Malaria remains a serious health problem in nine countries of the Western Pacific Region: China, the Lao People's Democratic Republic, Democratic Kampuchea, the Philippines, Malaysia, Papua New Guinea, Solomon Islands, Vanuatu and Viet Nam. The major technical problems are resistance of *Plasmodium falciparum* parasites to chloroquine and other drugs and resistance of major vector mosquitos to insecticides.

Parasitic diseases such as schistosomiasis, filariasis, and protozoal and helminthic infection continue to pose health problems. Following the effective use and wide acceptance of the new drug, praziquantel, there has been a change in the overall strategy for controlling *Schistosoma japonicum* infection, which is now aimed at reducing morbidity rather than halting transmission. The same drug has served to boost and revitalize national control programmes for other trematode infections such as paragonimiasis and clonorchiasis.

The distribution of leprosy in the Region presents marked differences between countries or areas and within a given country. WHO has been collaborating in the implementation of multidrug therapy to ensure proper treatment of cases, and in applied research on rapid diagnosis of *Mycobacterium leprae* infection in order to facilitate early diagnosis. It is hoped in this way to achieve rapid progress in the control of leprosy in the Region.

Diarrhoeal diseases are the second leading cause of morbidity and mortality in Democratic Kampuchea, the Lao People's Democratic Republic, Papua New Guinea and the Philippines. In China, Malaysia and the South Pacific, morbidity is high but mortality is decreasing. Bacteria such as *shigella, V. cholerae* and *Campylobacter* are still the main causes of diarrhoea; in recent years, however, several viruses, particularly rotavirus, have been recognized as important etiological agents in acute diarrhoeas of infants and young children. The WHO strategy of providing oral rehydration therapy has significantly contributed towards the reduction of mortality in children under five years of age.

Hepatitis B virus infection is highly endemic in many countries of the Region with carrier rates ranging from 2.7% to 11.0%. Primary hepatocellular carcinoma, which is closely associated with the disease, is the most common form of cancer in many of the developing countries. WHO is providing technical cooperation to China for the large-scale production of hepatitis B vaccine, which is quite effective in preventing the transmission of the virus. A system for the collection of high titred HBsAg plasma in the South Pacific has been established with the technical cooperation of WHO. The collected plasma will then be sent to Japan for processing into hepatitis B vaccine, for eventual use in the South Pacific.

Japanese encephalitis is endemic in China, Japan and the Republic of Korea. Its occurrence in epidemic form has been a cause of great concern in Viet Nam. Surveillance of the disease is

being strengthened, and development of a new vaccine using recombinant DNA technology is being explored.

Dengue fever/dengue haemorrhagic fever is present in China, Malaysia, the Philippines, Singapore, Viet Nam and countries of the South Pacific. A live attenuated dengue-2 vaccine has been prepared and is being tested in Thailand. Research on the preparation of a new dengue-2 vaccine using recombinant DNA technology is progressing in Australia, Japan and Malaysia.

Hantaan virus, the etiological agent of haemorrhagic fever with renal syndrome, is endemic in China and the Republic of Korea. Serological surveys on rodents collected in Hong Kong, Japan and Singapore have demonstrated the presence of antibodies to Hantaan virus, suggesting that the virus may be widespread in the Region.

The increasing prevalence of sexually transmitted diseases, notably gonorrhoea caused by penicillinase-producing strains of *Neisseria gonorrhoeae,* has been reported in various countries of the Region, and the possible resurgence of syphilis, due to the use of antibiotics other than penicillin in the treatment of penicillin-resistant gonorrhoea, has been of concern to many countries. It is proposed to initiate a surveillance programme on antibiotic resistance.

Cases of acquired immunodeficiency syndrome (AIDS) have been reported in the Region and it is feared that in due course it might become widespread. Simple diagnostic procedures are being developed to facilitate surveillance of the disease.

Symposium
Diarrheal diseases in tropical area

1 BACTERIAL DIARRHEAL DISEASES

YOSHIFUMI TAKEDA
Department of Bacterial Infection, The Institute of Medical Science, The University of Tokyo

Acute diarrheal diseases are recognized as a major cause of mortality and morbidity in children in developing countries in the tropical world. The World Health Organization estimated the number of deaths due to acute diarrheal diseases in children under 5 years old in 1980 as about 4.6 million in Africa, Asia (excluding China), and Latin America. Several enteric pathogens, such as *Vibrio cholerae* O1, *V. cholerae* non-O1, enterotoxigenic *Escherichia coli,* enteropathogenic *E. coli, Salmonella, Shigella, Campylobacter jejuni* and *V. parahaemolyticus* have been shown to cause acute diarrheal diseases.

The pathogenesis of these enteric bacteria has been a focus of research. Many toxins responsible for acute diarrhea have been identified and the physicochemical, immunological and biological properties of some of these toxins have been examined. The invasive character of some of these bacteria has also been studied and at a molecular level the plasmids encoding invasiveness have been identified.

Among the many toxins so far reported, cholera enterotoxin is the best understood. It stimulates membrane-bound adenylate cyclase of target cells and increases the intracellular level of cyclic AMP. Heat-labile enterotoxin of enterotoxigenic *E. coli* shows similar physicochemical, immunological and biological properties to those of cholera enterotoxin. Recently, the molecular structure of heat-stable enterotoxin of enterotoxigenic *E. coli* was characterized. This toxin stimulates membrane bound guanylate cyclase and increases the intracellular level of cyclic GMP, but not adenylate cyclase. The mechanism by which intracellular increase of cyclic nucleotides in the target cells causes significant secretion of water is still unknown.

Shiga toxin produced by *Shigella dysenteriae* has also been well characterized. Although the role of Shiga toxin in *Shigella* infection is not fully understood, the potent cytotoxic activity of Shiga toxin may somehow be related to dysentery caused by the organism.

Recently, some *E. coli* strains that belong to 0157: H7 were found to produce a toxin immunologically related to Shiga toxin. Further studies revealed that these strains also produce a potent cytotoxin that is immunologically unrelated to Shiga toxin. The roles of these toxins in *E. coli* 0157: H7 infection require further study.

2 VIRAL DIARRHEA IN THE TROPICS

CHIAKI MIYAZAKI
Department of Pediatrics, Faculty of Medicine, Kyushu University

Acute infectious diarrheal illness is recognized as one of the leading causes of morbidity and mortality in tropical developing areas. Viral agents discovered during the last decade have been proven to be responsible for a large population of diarrhea with defined etiologic agents. Especially, from numerous cross-sectional studies in more than 30 countries (including the tropics), rotavirus has been documented as a major etiologic agent for gastroenteritis in children under 2 years of age. Fifteen to seventy per cent of the hospitalised cases (especially cases with severe degree of dehydration) in these studies have been found to be associated with rotavirus. Studies in temperate climate areas revealed peak incidence of rotavirus diarrhea in children between 6 months and 2 years of age, while a good proportion occurs in infants under 6 months of age in developing countries. This discrepancy may due to an earlier exposure to contaminated environment under poor sanitation in developing areas. Rotavirus diarrhea shows its peaks of occurence in cooler seasons in temperate climate areas, compare to a year-through but with occasional outbreaks in tropical areas.

From 1982 to 1984, we studied epidemiology of rotavirus infection in Kenya in East Africa, and over 2,000 diarrheal stools from Kenyan infants and young children were collected at some hospitals. Under monthly surveillance, an average of 22%, range 8 to 50%, of the stools showed rotavirus-positive by ELISA. Age distribution of rotavirus diarrhea was similar to other report from tropical countries. By electropherotyping of viral RNAs, rotaviruses assigned to subgroup II were found predominant in Kenya (similar to other countries), and the alternation of predominant strain was observed in the studied area. Monthly detection rate of rotavirus did not correlate with climatological data.

Although therapy with oral rehydration solution succeeded in decreasing the mortality of severely dehydrated diarrhoic children in many developing countries, prophylaxis with vaccine would have a significant impact. Multiple divergent approaches have been undertaking to develop live oral vaccine against rotavirus: (1) rotaviruses of animal origin, (2) attenuated human rotavirus, (3) reassortant hybrid virus, etc. Several strains of animal origin including bovine strain RIT 4237, rhesus strain WWU 18006 and bovine strain WC 3 have been undergoing clinical trials for their safety, immunogenicity and effectiveness in several countries. In human rotavirus has two subgroups (I and II) and at least four serotypes. Human and animal rotaviruses usually share common group antigen. If heterotypic immunity to a given vaccine is found to be enough for protection against any human rotavirus serotype, these vaccine candidates could be truely useful. It should be noted that none of these vaccines will likely protect new rotaviruses lacking common group antigen which have recently caused large outbreaks of gastroenteritis in China. Anyway live vaccine may become a strong weapon against rotavirus diarrhea of infants and young children in the near future.

3 CURRENT CONCEPTS OF *ENTAMOEBA HISTOLYTICA* AND AMOEBIASIS

TSUTOMU TAKEUCHI
Department of Parasitology, School of Medicine, Keio University

In the present symposium, recent concepts of *Entamoeba histolytica* and its infection are discussed as a model of protozoan diarrheal disease, because numerous significantly novel knowledges have been obtained on amoeba and amoebiasis.

Amoebiasis is still one of the major health hazards in developing countries. According to WHO, there are 400,000,000 people annualy infected with this protozoan, most of whom are apparently located in the developing nations. On the other hand, the number of cases with this enteric infection had been dramatically decreasing from 1950 to 1975 in developed countries; accordingly, it seemed to be of no more significance in their public health. Since 1980, however, we have had a sudden increment in the number of cases with invasive amoebiasis in Japan. Our later investigation suggests that this is attributable to sexual transmission of this pathogen, at least partially, among biased males. The same pattern of its transmission also has been confirmed in the United States, the United Kingdom and Canada. Although some differences have been found concerning the factors which affect this mode of transmission between Japan and other developed countries, it is undoubtedly important in relation to AIDS.

New knowledges have also been obtained on the biology of *E. histolytica,* in particular on its pathogenesis. It is now evident that cell killing and subsequent phagocytosis are the primary mechanism of its pathogenicity. Current evidences suggest that there are two modes of cell killing by this protozoan, i.e., one is contact-dependent and the other contact-independent cell killing. The former is considered to be attributed, at least partially, to the action of "pore-forming proteins" which deloparized J224 macrophage-like cells and spleen lymphocytes, although they were not found on the plasma membrane of amoeba. On the other hand, the contact-independent process is made by a protein of molecular weight 35,000 to 40,000 and a pI 4.5 to 5.0. The activity of this protein is inhibited by N-acetylgalactosamine, suggesting a lectin-like property.

Concerning diagnosis of amoebiasis, serodiagnosis is certainly the primary measure. Currently, ELISA, CIE and gel diffusion are widely employed for this purpose. Indirect immunofluorescent antibody technique was found effective only for hepatic amoebiasis. However, the IFA developed in our laboratory was found as effective as gel diffusion and CIE for the serodiagnosis of both intestinal and hepatic amoebiasis. Moreover, of particular interest concerning this modified IFA was that it qualitatively agreed with gel diffusion at more than 97% when tested on asymptomatic cyst passers, suggesting that there are two types of carriers; one with and the other without tissue invasion of this parasite.

Chemotherapy of amoebiasis has been primarily done by nitroimidazole derivatives like metronidazole. However, distinct mutagenicity and carcinogenicity in experimental models, and appearance of metronidazole-resistant *Trichomonas vaginalis* indicate an importance of development of new anti-amoebic drugs. We have been testing some series of compounds, and found that dichlorophene, a halogenated bisphenolic derivative, had potent anti-amoebic effects. Some

other compounds of plant origin also have been found effective in treating experimental amoebic liver abscess.

4 STUDY ON THE SEROTYPING AND BIOTYPING OF 300 STRAINS OF *CAMPYLOBACTER* BY LIOR'S METHOD

SIMA HUILAN[1], XIE MEIWEN[2] AND XU JUFEN[2]

Consultant in Health Laboratory Technology, WHO Western Pacific Regional Office, Manila[1] and Shanghai Hygiene and Antiepidemic Center, Shanghai, China[2]

Three hundred *Campylobacter* strains isolated from human (277 strains) and animal (23 strains) were serotyped by Lior's slide agglutination assay. The typing sera were supplied by Dr. Lior through WHO Geneva.

The result showed that 251 strains (83.7%) were typable and 49 strains (16.3%) were untypable. Of the 277 human strains, 233 were typable and of the 23 animal strains 18 were typable. These figures represent 84.1% and 78.3%, respectively. The typable strains from human belonged to 31 serogroups and 16 sub-serogroups (agglutination in pairs of sera) of which the most common serogroups were serogroup 1, 2, 4, 9, 28, 32, 36, 48 and 60. More or less these figures are similar to those reported in European countries where, the most common serogroups are 1, 2 and 4. On the other hand, the very common serogroup 7 in Europe is not found in Shanghai, China.

According to a new extended biotyping scheme of Lior, 213 (76.9%) human strains belonged to *Campylobacter jejuni* I and II, 64 (23.1%) belonged to *Campylobacter coli* I and II, whereas all of the 18 animal strains (12 strains from swine) belonged to *Campylobacter coli* I and II. The result of serotyping and biotyping assay of 300 *Campylobacter* strains indicated that swine was one of the important sources of infection of the *Campylobacter* enteritis in Shanghai, China which compared with Skirror's report that *Campylobacter coli* occupied 5% only among *Campylobacter jejuni/coli* group.

A comparison of Lior's and Penner's serotyping method showed Lior's slide agglutination assay was simple, rapid and easy to perform in the field trail for epidemiological investigation. The autoagglutination often occurred in slide agglutination when saline suspension was used. It could successfully be eliminated through suspending the bacteria in phosphate buffered saline containing DNase.

The preparation of the typing sera of *Campylobacter* will be studied further in Shanghai Hygiene and Antiepidemic Center.

5 STRATEGIES FOR THE CONTROL OF DIARRHOEAL DISEASE IN THE ASIAN PACIFIC REGION

CHEV KIDSON
Queensland Institute of Medical Research, Brisbane, Australia

The patterns of diarrhoeal disease differ greatly from country to country in this region and indeed from one area to another within a given country. These differences relate to acute versus chronic diarrhoeas, to relative frequencies of particular etiological agents, to the degree of morbidity, to the extent of mortality and to the age group affected.

Given the differing spectra of geographical characteristics, population size, economic status, health care service accessibility, social organization and stage of development of national programs for the control of diarrhoeal diseases (CDD), this variability is hardly surprising. However, the practical implication is that it is essential to have a flexible approach to achieving the goals of the global CDD program.

In countries such as the People's Republic of China the mortality from acute diarrhoeal disease is low, whereas in countries such as the Socialist Republic of Vietnam or Bangladesh there is still significant mortality. The differences relate to both disease patterns and to accessibility to appropriate health services. Thailand, for example has established an effective national CDD program, as has the Philippines, but these countries have had differing success thus far in reducing mortality due to acute diarrhoea.

Morbidity data are not readily available for many countries but morbidity may be high even when mortality is low, e.g. in China. This type of situation usually reflects adequate treatment services but inadequate organization of preventive programs at this time.

In general in this region, as elsewhere in, the world, there is a correlation between the use of oral rehydration therapy and reduced mortality in acute diarrhoea. There is generally a high association of rotavirus, *E. coli* and a number of other organisms with acute diarrhoea in children. Indeed, the similarity in etiological patterns is remarkable, suggesting that common strategies may be applicable for prevention. However, there are some marked differences from area to area in the frequency of shigella and amebic dysentery which appear to be related to socioeconomic and behavioural variables of considerable consequence to the planning of preventive strategies.

A number of important research studies focused on environmental or social interventions are in progress in some countries in the region. Many more are needed to address the key issue of the economic implications of control strategies where the latter may impinge on longstanding agricultural or sanitaion practices. These strategies need to be weighed against other potential interventions involving technologies such as immunoprophylaxis using new vaccines.

Although epidemiological information on acute diarrhoea is far from complete in most areas, data on chronic diarrhoeas and their relationship with nutritional status are far less available. In this context there are important challenges for the development of molecular diagnostic tools suitable for epidemiological surveillance to permit more rational control strategies to be implemented. These developments need to be combined with socioeconomic analyses to facilitate planning towards achievable goals in the control of diarrhoeal disease in countries in the region.

Tsutsugamusi disease (scrab typhus) in asia

1 MEDICO-ZOOLOGICAL STUDIES OF TSUTSUGAMUSHI DISEASE IN JAPAN, WITH SPECIAL REFERENCE TO *LEPTOTROMBIDIUM PALLIDUM*

KIYOSHI ASAMURA
Department of Zoology, National Science Museum, Tokyo

An outbreak of tsutsugamusi disease or chigger-born rickettsiosis among American soldiers in the foothills of Mt. Fuji in 1948, established the existence of a hitherto unknown endemic area of the disease in Japan. This incident suggested also the occurrence of the disease transmitted not by *Leptotrombidium akamushi* (classical type of the disease) but by other species of chiggers (new type of the disease), and at present, *L. scutellare* and *L. pallidum* are determined as new vectors in certain areas in Japan.

A monthly occurrence and rickettsial positivity of *L. pallidum* and *L. akamushi* found on rodents, *Microtus montebelli* in a classical endemic area in Niigata Prefecture indicate seasonal difference to the case incidence of the disease in this Prefecture, i.e. the cases in spring as well as autumn are transmitted by *L. pallidum* whereas the cases in summer mainly by *L. akamushi.*

As regards seasonal occurence of *L. pallidum* in Japan, there are 2 different types according to localities; one is the type which has 2 peaks in late spring and autumn in northern parts of Japan, while the other type has a single peak from late autumn to winter or early spring in more or less southern parts of this country, such as Kanagawa, Shizuoka, Tokyo, Oita and so on. The monthly occurence and rickettsia positivity of *L. pallidum* in Oita prefecture seemed coincided with the case incidence of the disease therein.

Besides these 2 vector species of new type disease, the possible existence of other vectors can be assumed on the basis of man infesting habits in *L. palpale, L. pallidum burnsi, Neotrombica japonica, N. mitamurai* and others, and positive isolation of rickettsia from *L. kitasatoi, L. palpale, L. murotoensis, Neotrombicala pomeranzevi, Cheladonta ikoensis* and others.

The extraordinary occurence of cases of new type disease in the season when *L. pallidum* and *L. scutellare* are missing or a few in number, such as the cases in April in the foothills of Mt. Fuji and in summer in Kagoshima Prefecture, seem to be worthy of future investigation.

On the other hand, it should be noticed that in Hokkaido, no cases have yet been reported though the rickettsia was frequently isolated from rodents as well as a chigger, *L. kawamurai.*

2 STUDIES OF TSUTSUGAMUSHI DISEASE IN PENINSULAR MALAYSIA

AKIRA SHIRAI
WHO/WPRO, Manila, Philippines

Early studies initiated by the United States Army Medical Research Unit in Malaysia had shown that 23% of the febrile patients presenting at the district hospital had scrub typhus. Almost 80% of the cases were oil palm workers.

Since there was an indication that human infection rates might vary according to the stage of oil palm growth, studies were undertaken in central Peninsular Malaysia to determine the risk of infection in areas containing oil palm of different ages. Three settler populations, those settled for one year, 5 years and 8 years, were identified for the survey. Antibody prevalence rate was the highest in the individuals settled for 5 years. The impact of ecological changes on the population of scrub typhus vectors was also examined. A peak in the number of chiggers per host (*Rattus tiomanicus*) was noted in the area with 2–1/2 year old trees. Majority of the chiggers collected were *Leptotrombidium deliense.* In the older oil palm areas, *L. deliense* made up less than 1% of the chigger population. Most were *Ascoshoengastia indica,* a non-vector, nest-dwelling mite.

Infection rates in the chiggers, rodents, and humans were also determined from the isolation rates of *Rickettsia tsutsugamushi.* The rates were 4% in the chiggers, 11.7% in the rodents, and 45% from the febrile patients working in the corresponding areas containing younger trees. A much lower prevalence of infection was noted in an area with more mature trees, with only 0.6% in rodents and 0.1% in chiggers.

The scrub typhus vector population within the oil palm habitat can be directly related to the natural succession of the oil palm scheme. Burning the forest eliminates most chiggers and their hosts from the ground surface, leaving only pockets of infestations within small unburned areas or in underground nests. As the grasses under the covercrop begin to grow, a suitable habitat for the vector chiggers and their mammalian hosts is formed. The hardy *R. tiomanicus* takes over as the dominant mammal within the oil palm habitat. The vector population continues to build up until the time of harvesting and the arrival of settlers. Over a period of a few years, the thick grasses between the trees will be partially eliminated by cultural practices of the settlers, but harborage for *R. tiomanicus* will be formed by the placement of palm fronds between the oil palm rows. As the trees continue to grow and the canopy closes, the vector habitat is again diminished and the hosts begin to form nests in the little piles and/or the oil palm trees. The area between the tree does not contain suitable grasses to maintain a Trombiculid chigger population.

3 EPIDEMIOLOGICAL FEATURES OF TSUTSUGAMUSHI DISEASE (SCRUB TYPHUS) IN SOUTHERN CHINA

LIN QUANSHENG
Department of Infectious Diseases, Jinan University
Medical College Guangzhou, China

Tsutsugamushi disease was recognized etiologically in Guangzhou in 1948 and subsequently in South-east South-west parts of China. In Southern China, cases occur with seasonal peak in July-Aug. Incidence appears to be greatest between 21 and 40 years of age which depends on the frequency of intrusion into the enzootic cycle. Eschar is seen in 84 to 98.2% of cases. Mortality in preantimicrobial therapy years varied from 16% to 61.4% which decreases markedly after administration of specific antibiotics. At least 18 species of Rodentia and Insectivora have been demonstrated to harbor *Rickettsia tsutsugamushi* in nature. A few species of larvae mites other than *L. deliensis* (the principal vector) are naturally infected. Presence of minor immunological difference in local isolated rickettsia strains which should be considered in vaccine development.

4 ELECTRON MICROSCOPIC STUDIES OF INTRACELLULAR MULTIPLICATION OF *RICKETTSIA TSUTSUGAMUSHI*

AKIRA TAMURA
Department of Microbiology, Niigata College of Pharmacy

The mechanism of penetration of purified *Rickettsia tsutsugamushi* (Gilliam strain) into cultured mouse fibroblasts (L-cells) and the profiles of the rickettsial growth in the cells were investigated by electron microscopic observation of the specimens prepared at various times of infection. After 10–40 min of infection, rickettsiae in the process of being phagocytized were often seen on the cell surface. These were restricted to the rickettsiae which seemed to be intact in morphology, while heavily plasmolyzed ones were never phagocytized. Additionally, rickettsiae were taken up individually into a phagosome, and phagocytosis of several rickettsiae together was rarely observed. In the cells, phagosomes whose membranes enclosed rickettsiae either tightly or loosely were seen. Rickettsiae in the loose phagosomes often showed signs of plasmolysis and were rarely released into the cell cytoplasm. Partial disintegration of phagosomal membranes and the escape of only morphologically intact rickettsiae from the phagosomes were seen only in tight phagosomes. Large phagosomes containing a clump of several rickettsiae were observed occasionally, in which case the microorganisms were deformed and seemed to be denatured. From the above observations and the frequency of appearance of these different penetration stages in the specimens of 10, 20 and 40 min after infection, it was concluded that the rickettsiae enter initially into a tight phagosome by phagocytosis and are then released into the cell cytoplasm by disruption of the phagosomal membrane. No other

mechanisms of penetration were found.

Although the process of penetration of rickettsiae into host cells proceeded within relatively short period of 10–40 min after infection, multiplication of the microorganisms in the cells were slow and the observations were performed at day-intervals. At the early stage of infection (24 hr of infection), many of the rickettsiae in host cell cytoplasms showed electron-less dense inner structures, but electron-dense type became dominant at the later stage of infection (72–120 hr). Binary fission of rickettsiae by forming a constriction at the middle of long axis was often observed at the middle stage of infection (72–96 hr) in the cytoplasm or at the periphery of the host cells. Although many rickettsiae were 1.2–2.5 μm in length and 0.5–0.8 μm in width, abnormally long rickettsiae, 10–15 μm in length, were sometimes seen. Additionally, intranuclear proliferations of this rickettsia were observed, but the incidence was very low. In the specimens prepared after 72 hr of infection, release of the microorganisms were seen at the host cell surface by lifting up cell membrane from inside to outside, like as the budding profile of enveloped viruses. The budding rickettsiae increased in number with the lapse of time. In a thin section of the later stage of infection (96–120 hr), detachment of the outer and inner leaflets of the host cell nuclear membrane and formation of vacuoles were observed. Then the cells were round up and, in some cells, the cytoplasmic membrane disappeared and rickettsiae inside the cells were released into the culture fluid.

5 RAPID SEROLOGIC DIAGNOSIS OF TSUTSUGAMUSHI DISEASE BY MEANS OF INDIRECT IMMUNO-PEROXIDASE (IP) TECHNIQUE

TSUNEHISA SUTO

Department of Microbiology, Akita University School of Medicine

Tsutsugamushi disease had been recognized as a serious disease in Japan for more than one handred years. However, over the years the number of cases has been steadily declining with only a few cases being reported during the years of 1965 to 1975. The disease seemed to have been nearly eliminated.

Since 1976, however, the reported cases of the disease began to increase in many parts of the country, even where the disease had never been identified previously. The disease appears to be transmitted by *Leptotrombidium pallidum* in the early summer and autumn months and by *Leptotrombidium scutellare* during the winter months.

This significant increase in the number of reported cases of Tsutsugamushi disease in Japan has occured concurrently with the wide spread use of beta-lactam antibiotics which are not effective in treating Tsutsugamushi disease. The use of these antibiotics led to the occurence of several fatal cases of Tsutsugamushi disease which were confirmed by a specific serological examination following death.

These unfavorable occurence have served as a stimulus to develop a rapid and accurate serologic diagnostic procedure employing the indirect immuno-peroxidase (IP) technique. This technique is performed on a glass slide using minute dots of antigen prepared by concentrating particles of rickettsia grown in L-cell cultures.

An accurate diagnosis can be made within a few hours after receiving the serum specimens

by observing the titers of the specific IgG and IgM antibodies in the serum of the patient who is suspected of having the disease or has a fever of unknown origin.

Using this procedure we have detected a total of 465 cases of Tsutsugamushi disease, from 19 prefectures, out of 1,368 cases of suspected disease or in cases with a fever of unknown origin or in patients with exanthema of unknown cause during the 6 year period from May 1980 through June 1986.

In the 7 patients that expired the direct cause of death was disseminated intravascular coagulation (DIC) syndrome. They were all confirmed seropositive for Tsutsugamushi disease by means of our IP method within a day of arrival of the serum specimen which was collected at a variable periods before death. The most patients responded rapidly after the correct diagnosis was made and adequate treatment with tetracycline or chloramphenicol was initiated.

This IP procedure is proposed as the most efficient method for rapid and specific diagnosis of Tsutsugamushi disease at present time in Japan, where beta-lactam antibiotic therapy is commonly used as initial treatment of fevers associated with infectious diseses.

General presentation

1 STUDY OF QUICK AND SIMPLE STAINING FOR MALARIAL PARASITE — APPLICATION FOR SCREENING OF MALARIAL PARASITEMIA AND DETERMINATION OF DRUG SENSITIVITY TO MALARIAL PARASITE

YOSABURO OIKAWA[1], ELYUSRAR ALYUSAR JALAL[2] AND SHOKICHI TANI[3]

Department of Medical Zoology, Kanazawa Medical University[1], Institute of Tropical Medicine, Kanazawa Medical University[2] and Health Care Center, Fujitsu Kawasaki Hospital[3]

We previously reported at the 55th annual meeting of the Japanese Society of Parasitology about new method for staining of malarial parasite (*Plasmodium berghei*). This method was more simple and quick than the Giemza's stained blood film method in the case of malarial parasitemia count. The coefficient of correlation between the malaria infected red blood cell count by this method and the blood film method was more than 0.9. In this report, we studied the application of this method for the screening of malarial parasitemia and the determination of drug sensitivity to malarial parasite.

Material and method: For the screening, Gentian Violet Stock Solution (Wako) was diluted 50 times by 10% methanol and 0.3% EDTA-2Na contained 0.85% saline. The blood of malaria infected mouse was dropped on the head of a finger, until the diameter was about 3 mm, and placed it on the slide glass (about 0.008 m*l*). A staining solution was dropped on the blood, mixed and covered with a cover glass (24×50 mm). Malarial parasite in red blood cells were stained dark violet and observed like a ball. When the parasitemia was about 0.1%, malaria infected red blood cells were discovered easily by this method. For the test of drug sensitivity, Gentian violet stock solution was diluted 400 times by 10% methanol and 0.15% EDTA-2Na contained 0.85% saline. The in vitro-cultured blood was diluted 5 times by this staining solution and the parasitemia was counted with Bürker-Türk hemocytometer. At the study of the sensitivity for quinine dihydrochloride, the decrease of the parasitemia was observed clearly on the second day of culture with candle jar by this staining method, and the parasitemia count was similar with the result from the blood film staining method.

Consideration: Giemza's staining blood film method is necessary for the discrimination of malarial species, but it takes some hours for making the preparation and scanning the sample microscopicaly with immersion oil. In new staining method, the preparation making and the sample scanning were easy, because the scanning was enough at ×400. So the new method cosidered to be more useful for the screening of malarial parasitemia at the site of the mass treatment in the malaria endemic area instead of the blood film staining method. In the test of drug sensitivity for malarial parasite, the new method was also useful for the observing of the reduction of parasitemia.

2 ISOLATION AND SOME PROPERTIES OF PROTEOLYTIC ENZYMES FROM *PLASMODIUM BERGHEI* INFECTED MICE ERYTHROCYTES

FUMIHIDE INOUE[1], SEIKICHI TOKU[1], REIKO MATSUYAMA[2] AND YOSHIYA SATO[3]

The First Department of Biochemistory[1], RI Experiments Facility[2] and Department of Parasitology[3], School of Medicine, University of the Ryukyus

An aminopeptidase was purified from mouse erythrocyte membrane infected with malaria parasites (*Plasmodium berghei*, NK-65 strain) by a simple method involving selective extraction with non-ionic detergent Triton x-100 and chromatography on Sephadex G-100 and DEAE-Sephadex A-25. This enzyme has an apparent molecular weight of about 100,000 as measured by gel filtration. Activity was monitored conveniently with L-alanine-p-nitroanilide or L-leucine-p-nitroanilide as substrate at 405 nm. The enzyme was inhibited by bestatin and chymostatin but not by leupeptin, phosphoramidon, antipain, pepstatin and E64. The aminopeptidase has an essential sulfhydryl group at the active site which is rapidly modified by Hg^{2+} and slowly modified by p-chloromercuribenzoic acid, but is unaffected by monoiodoacetic acid, or N-methylmaleimide.

3 KINETIC CHANGES OF HEMOPOIETIC STEM CELLS IN *PLASMODIUM BERGHEI*-INFECTED MICE

MEIKO ASAMI, HARUHIKO MARUYAMA, MAKOTO OWHASHI AND YUKIFUMI NAWA

Department of Parasitology, Miyazaki Medical College

Drastic hematological changes are observed during malaria parasite infection because of the destruction of red cells and activation of immune as well as mononuclear phagocyte system. Although all these blood cells are originated from common precursor, termed hemopoietic stem cells, not much attention has been payed for the changes of hemopoietic stem cells during malaria infection. In the present study, therefore, kinetic changes of hemopoietic stem cells were examined in *Plasmodium berghei*-infected mice.

The number of hemopoietic stem cells in the bone marrow was measured by 2 different assay systems: in vivo spleen colony assay, which can detect pluripotential stem cells (CFU-S), and in vitro soft-agar colony assay, which can detect committed stem cells (CFU-C) towards granulocyte and macrophages.

The number of CFU-S started to increase on day 4, reached a peak on days 6-8, and then decreased to subnormal level by day 14. The kinetic change of CFU-C was essentially the same as that of CFU-S with slightly delayed onset.

In addition to in vivo and in vitro colony assays, ^{59}Fe uptake in bone marrow and spleen was

also examined. Iron uptake in the bone marrow of infected mice was twice and that in the spleen of infected mice was seven times higher than that in the respective organ of uninfected mice.

These results indicate that hemopoietic stem cells are activated during an acute stage of *P. bergehei* infection.

4 CHANGES OF T-LYMPHOCYTES AFTER BABESIA INFECTION IN MICE PRETREATED WITH TOXOPLASMA LYSATE ANTIGEN (TLA)

NAOYOSHI SUZUKI[1], SHINICHI SEKIGUCHI[1], MOTOYOSHI SATO[1], HARUHISA SAKURAI[1], ATSUSHI SAITO[1], TSUNEO HIROSE[1] AND FUMIO OSAKI[2]

School of Veterinary Medicine, Obihiro University[1] and Kobe City[2]

With the changes of lymphocytes in the thymus, spleen, liver and blood were examined both biochemically and histopathologically in mice treated with TLA before inoculation with *Babesia rodhaini* which was fatal to rodents. The number of Thy-1 positive (+) cells in untreated mice was 3.7×10^7 cells in the whole thymus 1 day before inoculation (b.i.) while those in TLA-treated mice were 6.5×10^7 and 0.9×10^7 cells 1 day b.i. and 10 days after inoculation (a.i.), respectively. The total number of Thy-1 (+) cells in the spleen was 3.9×10^7 and 11.7×10^7 1 day b.i. and 10 days a.i., respectively in untreated controls and 12.9×10^7 and 15.6×10^7, respectively in TLA-treated mice. A remarkable increase of Thy-1 (+) cells in the liver of TLA-treated mice 10 days a.i. was almost 10 fold as compared with that of 1 day b.i.. The increase of Thy-1 (+) cells in the liver in TLA treated mice was found around perivascular ducts 10 days a.i., however, showing few Thy-1 (+) cells around the ducts in untreated mice. These Thy-1 (+) cells around perivascular ducts were mainly $Lyt^{1,2}$ positive and $Lyt^{2,3}$ negative cells. Increase of Thy-1 (+) cells in the liver and spleen and decrease of those in the thymus are presumably owing to T-cells released from the thymus and flocked to the organs through the blood circulation.

5 MALARIA CONTROL IN NORTH SUMATRA, INDONESIA (1) GAMETOCYTOCIDAL EFFECT OF PRIMAQUINE

AKIRA KANEKO[1,2], KISEKO KAMEI[1,3], AKIRA ISHII[1,4] AND TAKESHI SUZUKI[1]

North Sumatra Health Promotion Project, JICA[1], Department of Parasitology, Hirosaki University School of Medicine[2], Department of Parasitology, Teikyo University School of Medicine[3] and Department of Parasitology, Okayama University School of Medicine[4]

Considering the chemotherapy against falciparum malaria patients in its endemic area, it is important not only to clear trophozoits as clinical cure, but also to clear gametocytes which are

infective to vector mosquitoes. During passive case detection at Kuala Tanjung and Nana Siam, Sep.–Nov. 1985, 131 falciparum patients were treated by Tx: Fansidar 1,575 mg 1x, 87 were treated by Tx: Fansidar 1,575 mg+Primaquine 45 mg 1x.

After drug administration, these falciparum patients were followed up to observe the gametocyte fluctuation. During this study, trophozoites were cleared by day 7 in all of these falciparum cases without recrudescense.

In the group treated only by single Fansidar, 25 cases were examined on day 0 and day 2, and 99 on day 0 and day 7. In both subgroups, no significant change was observed in gametocyte load between day 0 and day 2 or day 7.

In the group treated by the combination of Fansidar and Primaquine, 29 were examined on day 0 and day 2, in which no significant change of gametocyte load was observed. But 72 cases, examined on day 0 and day 7, showed 73.6% gametocyte positivity rate (GR), 2.2 gametocyte density (PDI) on day 0; and 33.3% GR, 1.3 PDI on day 7. The difference was significant by Wilcoxon's test, ($p<0.01$).

The gametocyte-positive cases on day 7 were tried to be followed up weekly till gametocytes disappeared. In 52 cases treated only by Fansidar, 71.1% GR on day 0 continued till week 2, after that it declined but still as high as 11.5% in week 5. In 56 cases treated by Fansidar+Primaquine, 76.7% GR on day 0 had already declined to 30.3% on day 7, after that GR declined further to 7.1% in week 3.

This study showed the treatment of single Primaquine 45 mg together with schizontocide was effective in promoting the gametocyte clearance of falciparum malaria patients.

6 MALARIA CONTROL IN NORTH SUMATRA, INDONESIA (2) ENTOMOLOGICAL STUDIES AND VECTOR CONTROL

TAKESHI SUZUKI[1], TETSUSHI KIKUCHI[1], MASAHIRO TAKAGI[1,2] AND MASAYUKI YASUNO[1,3]

North Sumatra Health Promotion Project, JICA[1], Department of Medical Entomology, National Institute of Health[2] and National Institute for Environmental Studies[3]

The vector of the coastal malaria in our study area is *An. sundaicus*, which can not be controlled by DDT residual spraying, because of its exophilic nature. Based on this, studies have been concentrated on integrated control measures of the vector at its larval stage. A trial operation was started in a 32 km^2 area in September 1986.

The lagoons scattered behind a beach sand bank were infested heavily with the larvae. They were controlled effectively by draining of the water through a channel leading to a nearby river. Taking advantage of the fact that its breeding is limited only in sunny ponds, a control trial was done with success by shading of the ponds with coconut leaves. Biological control was done by releasing guppies, *P. reticulata*, into the ponds or ditches which were unable to avail source reduction/environmental management measures. Temephos was also applied in the breeding sites with the dose of 1 ppm, which was effective for 2 weeks.

Based on the information obtained through such trial operation, a large-scale control

operation is now under planning, aiming at the eventual build-up of vector control measures to be utilized by villagers themselves in future under community participation.

7 EXTREMELY HIGH TRANSMISSION OF MALARIA AMONG THOSE WHO LIVE NEAR BREEDING SITES OF THE SEA-WATER MOSQUITO, *AN. SUNDAICUS*, IN THE COASTAL AREA OF NORTH SUMATRA, INDONESIA

HIDEKI ITOKAWA
Department of Medical Zoology, Saint Marianna University, School of Medicine

A mass-blood examination was undertaken in the coastal area of North Sumatra, Indonesia, as one of activities on the project for the promotion of health. The aim for the survey was to determine the endemicity among persons who lived near breeding sites of the responsible vector, *An. sundaicus*. Very high parasite rates (around 60%) were obtained in young age groups (0–9 years). On the other hand, the parasite rates in adults showed relatively low (10–20%). Also, high parasite density cases were found only in the young age groups. These findings means that the existence of high-level immunity against malaria among the examined persons.

Salivary gland dissections were performed on 341 *An. sundaicus*, which had been collected from the same area, and 2 positive cases were obtained (0.6%). This sporozoite rate means that *An. sundaicus* brings about high transmissions of malaria in the study area, where very high mosquito-densities of *An. sundaicus* (more than 30/man/hour) were usually obtained.

8 DOES *TRYPANOSOMA BRUCEI GAMBIENSE* CHOOSE FEEDER-CELLS?

PETER J. MHANDO, TETSUO YANAGI, SHUSUKE NAKAZAWA,
TOSHIHIDE FUKUMA AND HIROJI KANBARA
Department of Protozoology, Institute of Tropical Medicine, Nagasaki University

In the cultivation of *Trypansoma brucei gambiense*, newly prepared newborn mouse brain and muscle derived cells could support the growth of the organisms. They were also available for the cultivation of the new isolate of Tg. On the contrary established newborn mouse brain and muscle derived cells lost their supporting ability. Supporting cells and non-supporting cells were seeded on the one half area and the other half of a culture dish respectively prior to the inoculation of Tg. The parasites increased only on the area of supporting cells. It was reported that rapid growing cells could not support continuous growth of trypanosomes. Thus, supporting cells and non-supporting cells were exposed to the minimum X-irradiation to suppress their growth but no change was noticed in their supporting abillity for Tg. The cultured trypanosomes were morphologically identical to bloodstream forms and retained infectivity for

mice. Growing Tg showed nest-like clusters in intercellular space. This suggested the necessity of close contact of Tg with cells rather than soluble growth factor from them.

9 ANTI-PARASITE ACTIVITY OF CARBOCYCLIC INOSINE AGAINST AMASTIGOTES OF *LEISHMANIA DONOVANI*

HIROYASU SATAKE[1], OSAMU HIRAOKA[1], YUSUKE WATAYA[1], JULIETA Y. KIMURA[2], KAZUHISA MORISHIGE[2], TOSHIKI AJI[2], AKIRA ISHII[2], AKIRA MATSUDA[3] AND TOHRU UEDA[3]

Faculty of Pharmaceutical Sciences, Okayama University[1], Department of Parasitology, Okayama University Medical School[2] and Faculty of Pharmaceutical Sciences, Hokkaido University[3]

A promising approach for the development of chemotherapeutic agents possessing selective toxicity is the one based on certain unique features in structure or function of the parasite that differ from those of the host cells. *Leishmania* are incapable of synthesizing purines *de novo* and are thus dependent on the host for the source of preformed purines. This qualitative difference in the enzymes of purine salvage and interconversion pathways between host and parasite suggests a rational approach towards the design of agents selectivity toxic in the parasite. We reported that carbocyclic inosine is a potent inhibitor of promastigotes of *Leishmania* but significantly less toxic to mammalian cells.

In the present experiments, J774·1, a mouse macrophage line, was cultured in RPMI 1640 medium containing lipopolysaccharide. The adherent cells were exposed to promastigotes of *L. donovani* for 1 day, and treated with the drug. On the other hand, the macrophages were pretreated with the drug for 2 hours, and were exposed to promastigotes of *L. donovani.* Drug activity is then assayed by counting the number of infected host cells/100 host cells. The results indicate that carbocyclic inosine is active against amastigotes of *L. donovani in vitro.*

10 THE PREVALENCE OF *BLASTOCYSTIS HOMINIS* INFECTION IN HUMANS IN KYOTO CITY

MINORU YAMADA, YOSHITSUGU MATSUMOTO, TATSUYA TEGOSHI AND YUKIO YOSHIDA

Department of Medical Zoology, Kyoto Prefectural University of Medicine

For several decades, *Blastocystis hominis* Brumpt, 1912 has been considered to be a harmless yeast in the human intestine. However, Zierdt and his colleagues emphasized that it was not a yeast but a protozoa, though its taxonomic position was not established. In later studies *B. hominis* was receiving increased attention as a cause of diarrhea. The present study demonstrates the prevalence of *B. hominis* infection in Kyoto City, Japan, between June 1983 and July 1986. Twelve hundred and fifty-one persons who submitted stool specimens for

parasite examination were divided into 5 groups. Group 1 consisted of 14 foreign tourists. Most of them had visited South East Asia, India and Africa just before coming to Japan, and more or less complained of digestive trouble. Group 2 consisted of 79 patients who were admitted to Kyoto Prefectural University Hospital with digestive trouble. Group 3 consisted of 304 inpatients and 696 outpatients in this hospital without digestive complaint. Group 4 consisted of 122 mentally deficient persons in 2 private care facilities, and Group 5 were 36 staff members of those facilities. The stools were first examined for *B. hominis* by iodine-stained direct smear method, then confirmed by phase contrast microscopy and Giemsa stain. The results are as follows: In Group 1, 11 of 14 tourists (78.6%) were positive for *B. hominis*. In Group 2, 3 of 79 patients (3.8%) had *B. hominis*. In Group 3, 12 of 1,000 inpatients and outpatients (1.2%) had *B. hominis*. In Group 4, 21 of 122 mentally deficient persons (17.2%), and in Group 5, one of 36 staff members (2.8%), were positive for *B. hominis*. Of the 48 persons positive for *B. hominis* in total, 30 had other parasites, for example *Giardia lamblia, Entamoeba coli, Enteromonas hominis, Iodamoeba buetschlii, Ascaris lumbricoides, Trichuris trichiura, Necator americanus, Clonorchis sinensis, Metagonimus yokogawai, Schistosoma mansoni,* and *Echinostoma* spp.. It was noticed that *B. hominis* was found not only in loose and soft stool but also in formed stool. In conclusion, careful examination may reveal *B. hominis* still existing among Japanese people, especially in mentally deficient persons isolated in the closed facility. Furthermore, it is interesting to note that foreign visitors are strongly contaminated with this parasite.

11 A SERIOUS CASE OF AMEBIASIS ACCOMPANIED BY EXTENSIVE COLIC ULCER AND ABSCESS

TOSHIO NAKABAYASHI[1], TAKESHI NAKAMURA[2] AND KATSUMI AMIOKA[2]

Department of Protozoology, Research Institute for Microbial Diseases, Osaka University[1] and Nakamura Hospital, Hirakata, Osaka[2]

The patient was a 48 years old male resident of Osaka and had been hospitalized with schizophrenia for almost 20 years. He had no experience of oversea travel. When he was admitted with head trauma in Nakamura Hospital on February 10, 1986, a right abdominal tumor was noted.

On June 22 of the same year, he was admitted again with ileus symptoms accompanied by abdominal distension and cicatrical hernia on the abdominal wall. Constipation and remittant fever were noticed. Romanoscopy revealed neither redness nor ulcer on the intestinal mucosa. By X-ray examination, gas retention with passage disturbance was observed in the colon. The laparotomy was carried out on June 27. The ascending colon was stenotic with necrotic portions and retroperitoneal abscess was found along the colon. The cecum and the right flexture adhered tightly to the retroperitoneal wall and the liver, respectively. The intestine between the cecum and the middle part of the transverse colon were resected and anastomosed.

After the operation, the patient did not turn better and the level of consciousness became reduced. On July 4, many trophozoites of *Entamoeba histolytica* were detected in the sections of the resected colon. Next day, antiamebic chemotherapy was started with dehydroemetine (40 mg, i.m., once a day for 7 days) and metronidazole (500 mg per day, injected through

stomach tube for 7 days). However, the therapy showed no effect but the patient died on July 14, 17 days after the operation.

The mucosa of the resected colon was necrotic and covered with a thick yellowish pseudomembrane. The ulcer reached the adventitia with some foci of perforation. Numbers of *E. histolytica* all of which were trophozoites, phagocytizing erythrocytes, were found in the ulcerative foci and even in the deep layer of colon. The case was undoubtedly of an internal infection of amebiasis of which the accurate diagnosis was just made by tissue examination of the resected colon after the operation.

12 SEROLOGICAL DIAGNOSIS OF AMOEBIASIS BY MICRO-ELISA

HISASHI YAMAURA AND RYUKOH SHIRASAKA
Department of Parasitology, Tokyo Women's Medical College

In the present study, the reliability of the technique of enzyme-linked immunosorbent assay (micro-ELISA) for the detection of amoebiasis was examined. For the assay, peroxidase-conjugated anti-human IgG (Miles-Yeda) and ABTS, 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) were used as the conjugate and substrate, respectively. Serum samples from 23 patients of amoebiasis and 200 healthy residens (amoeba cyst negative) were diluted at 1 : 200 and examined.

The results of maximum absorbance among normal control sera was 0.181 (0.028–0.181), and 0.410 (0.410–1.820) at the minimal absorbance among amoebiasis. Hence, the absorbance of 0.2 was considered to be the highest value of negatives above which a case would be positive for amoebiasis or highly suspective.

Sera from 32 cyst passers examined, only 2 or 6.3% were positive for the reaction.

The results indicated that micro-ELISA technique with ABTS developed in the present study is applicable to the survey of amoebiasis.

13 USE OF WING SOUND TRAPS FOR MALE MOSQUITOES OF SOME VECTOR SPECIES IN FIELDS

TOZO KANDA AND KENICHI OGAWA
Department of Medical Zoology, St. Marianna University

Vector control of malaria is crucially important than any other method, such as drug administration, immunization of communities to protect from malaria transmission. It is similarly important in a certain endemic area of subperiodic malayan filariasis, such as Taiping, Malaysia, where the achivement of vector control became indispensable. Because existence of animal reservoirs of this filaria, one soure of infection still remains its endemicity despite DEC (diethylcarbamadine) administration to human parasite carrier.

Meanwhile, in the vector control the limitation of the use of insecticides is requiring to

promote the developments of control methods other than insecticides, such as physical, biological and biochemical control methods. Among various biological characters by species, taxon or population some differences of specific characters influence or relate to their mating behaviour or activity in their genetic divergence in nature. Thus the finding of difference in wing-beat frequencies among sibling or very closely related populations, one of sexual isolation mechanism in genetic divergence has been proved and utilized to collect or trap mosquitoes.

Due to difficulty to find or promote swarming of some vector species of malaria and filariasis in nature, wing sound trapping with some kinds of biting attractants and/or stimulants was performed around their breeding places at biting time after sunset. The experiment was carried out by using 3 kinds of attractants and/or stimulants using alone or in combinations. These three were wing sound trap, an animal in screened cage, and dry ice. When the sound having a frequency close to that of wing-beat of the same taxa of species was used. The most effective trap was a combination of a fitted frequency of wing sound with animal dry ice. For the specties of genus *Mansonia* the sound of 330 Hz to 350 Hz combined with above two attractants were able to collect 870 males and 839 males within one hour from 15 minutes after sunset. For *Aedes albopictus* the sound of 480 Hz with animal and dry ice was most effective, whereas the numbers of males captured varied greatly according to the time of trapping. The trap used for *Aedes* and *Mansonia* was a plastic cylinder having a speaker set in the middle of tube and adhesive plastic sheets covering inner surface at both ends of the tube, however, for *Anopheles* a plastic lantern covered with a polyvinyl bag having the glue spread on its surface was more efficient to trap mosquito than the former type of trap.

The reduction of the number of male mosquitoes from the population of *Mansonia* was achieved more remarkably by the repetition of trapping for several times at the breeding area than setting traps in the biting area by females, such as houses which were far from breeding places. The age of male *Mansonia* was determined by checking mesothoratic furcum. The analysis of the results of trapping and of age composition gave the information of the size and dynamics of population and daily production of both sexes of *Mansonia.* The trapping method mentioned earier was proved to be useful in the ecological investigations as well as for the development of control method without use of insecticides, and future integrated control methods.

The trapping method was also applied to investigations of population dynamics of Genus *Mansonia* and *Culex tritaeniorhynchus* at a rice field, Ban Pa Inn, Ayutaya, Thailand. The trapping surveys have been performed for once a month from May 1986 up to date. Highly prevalent months of both mosquitoes were from June to September, especially August was the highest in the number of trapped males of Genus *Mansonia,* whereas their females trapped were not many. In this plain swanpy area there were many breeding focuses where male mansonias trapped were constantly more than others.

The trapping of *C. tritaeniorhynchus* proved 2 peaks in its numbers, one was in May and another was in August, though their trapped numbers were not significantly high. The use of biting attractants, animal in cage and dry ice trapps collected more in the number of females of *C. tritaeniorhynchus.* The trapping system of wing-sound and biting attractants was effective both for physio-biological control of *Mansonia* and for survey of population dynamics of *Mansonia, Culex tritaeniorhynchus* and *Anopheles.*

14 PATHOLOGICAL STUDY ON MOSQUITO-BITTEN WOUND (III) AN IMMUNOLOGICAL STUDY

ELYUSRAR ALYASAR JALAL[1,2], TERUAKI IKEDA[1], KIICHI SATO[2] AND SUSUMU HOTTA[2]

Departement of Medical Zoology[1] and Institute for Tropical Medicine[2], Kanazawa Medical University

Guinea pigs were sensitized by biting to *Aedes aegypti* 2 times per week. Mast cells degranulation response and homocytotropic antibody response during the sensitization period were investigated. In addition, the ability of serum to passively transfer the sensitivity was also investigated.

The degranulation of peritoneal mast cells from guinea pigs sensitized by mosquito biting significantly increased from the 3rd week, and then even though fluctuated, the percentage of degranulation remain high until the 12th week. Homocytotropic antibodies measured by 24-hour PCA showed peak response on weeks 7 to 9, while homocytotropic antibodies by 8-day PCA showed a high response only on week 8. Serum IgG against mosquito antigen showed relatively low level as measured by ELISA, but specific IgG level increased slightly during weeks 5 to 8.

Normal guinea pigs injected intravenously with 1 m*l* of 8-day PCA positive serum showed hypersensitive skin reaction on the challenge with the mosquito bites 8 days after injection. The same serum previously heated for 30 minutes at 56°C showed less wheal diameter of skin reaction. Guinea pigs injected with normal serum showed no skin reaction.

The above results were summarized that: 1) At the similar time with the appearance of the skin reactions, the percentage of mast cells degranulation increased. 2) The mosquito bites induced the production of homocytotropic antibodies, mainly of IgE class. 3) Serum from sensitized animals can passively transfer the sensitivity. These results proved that the skin reaction elicited by mosquito bites is an immediate allergic reaction.

15 THE INFLUENCE OF BLOOD SUCKING ON THE SUSCEPTIBILITY OF *AEDES ALBOPICTUS* AND *AEDES AEGYPTI* TO CHIK VIRUS

HIROSHI YAMANISHI[1], EIJI KONISHI[2] AND TAKU UENOYAMA[2]

Kobegakuin Women's Junior College[1] and Department of Medical Zoology, Kobe University School of Medicine[2]

Female mosquitoes of *Cx. tritaeniorhynchus, Ae. aegypti* and the Oahu and Hyogo strains of *Ae. albopictus* were dissected after appropriate days of infection with CHIK virus to quantify the virus amount present in head-thorax, midgut and other abdominal parts of the mosquito body. In *Cx. tritaeniorhynchus,* a non-susceptible control, the virus was present in the midgut until 3 days after feeding. A low susceptible *Ae. aegypti* were observed in 12% of midgut specimens.

The other part of abdomen and the head-thorax did not show detectable infectivity after the 1st day of infection. The Hyogo strain of *Ae. albopictus,* moderate in susceptibility, was observed in 60% of midgut specimens. The Oahu strain, highly susceptible, indicated that the virus was present at higher titers and at higher proportions. All midgut specimens were positive for infectivity. The susceptibility of midgut was especially important for virus multiplication. In *Ae. aegypti,* effect of feeding and infection on virus multiplication was examined.

16 THE STATE OF JAPANESE ENCEPHALITIS VIRUS ACTIVITIES IN OKINAWA MAIN ISLAND

MASAYUKI TADANO[1], TSUYOSHI ISHIMINE[1], YOSHIHIRO MAKINO[1],
TOSHIKAZU ANZAI[1], TOSHIHIKO FUKUNAGA[1]
AND HIDEO HASEGAWA[2]

Department of Virology[1] and Department of Parasitology[2],
School of Medicine, University of the Ryukyus

In order to investigate the prevalence of Japanese encephalitis virus (JEV) in Okinawa main island, 3 methods were conducted during a period of March 1985 through October 1986: (1) detection of JEV antibody in swine sera collected at two-week intervals at the slaughter house (2) collection of vector mosquitoes (*Culex tritaeniorhyncus*: CT) by light trap (3) isolation of JEV from field caught mosquitoes (CT). Serum antibody positive rate over 80% was observed during periods June-December 1985 and July-October 1986 in the northern area, July-October 1985 and May-October 1986 in the central area and August-September 1985 and August-September 1986 in the southern area. Fluctuations of antibody positive rate were observed in all 3 areas. Overall positive rates were 50% in the northern area, 47% in the central area and 30% in the southern area. In most cases, overall positive rate was not decreased to 0% throughout the year (except only once at June 11, 1986). Anti-JEV IgM class antibody was detected during a period April through October 1985 and May through October 1986. Total numbers of CT collected were 1,233 in the central area (March-December 1985) and 10,875 in the southern area (May-October 1986). One strain (from the central area) and 18 strains (from the southern area) of JEV were isolated from the pools of these mosquitoes. Due to the recent drastic decrease of rice field in Okinawa main island, the breeding sites of CT decreased, which resulted in the decrease of vector mosquitoe population. Pigfarms are becoming larger scale and locate remote from the mosquito breeding sites. So, JEV may circulate within narrow area. Thus resulted in the fluctuation of antibody positive rate. In the southern area, the site where mosquitoes were collected was closely located to the watercress field where clean water was always filling in. These fields (other than rice field) will become important CT bleeding sites in Okinawa main island.

17 MICRO-NEUTRALIZATION TEST OF DENGUE VIRUSES USING 96-WELL PLATE AND PEROXIDASE-ANTI-PEROXIDASE TECHNIQUE

TSUYOSHI ISHIMINE[1], MASAYUKI TADANO[1], TOSHIHIKO FUKUNAGA[1] AND YOSHINOBU OKUNO[2]

Department of Virology, School of Medicine, University of the Ryukyus[1] and Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University[2]

In South-east Asia, there are areas where Japanese encephalitis virus (JEV) and dengue viruses (DEN1–4), both belong to Flaviviridae, are concomitantly prevailing. In these areas, hemagglutination inhibition test for serodiagnosis is not applicable because of the cross-reactivity between these viruses. In this case, neutralization test (NT) can be useful. However, conventional plaque forming method needs more sample volume and takes longer incubation period. So, it is not practical for seroepidemiological study, where a lot of specimens have to be handled. Recently, rapid NT method using 8-chamber slide and peroxidase-anti-peroxidase method has been developed. However, it still has problem in handling many samples and costs expensive. Here, we developed micro-NT using 96-well plate and applied it to DEN1–4. High correlation was observed between the dilution of virus solution and the number of focus formed. The appropriate incubation period of DEN2 and DEN4 was 40 hr, while DEN1 and DEN3 was 56 hr. This method has an advantage over 8-chamber slide method in that (1) more samples can be handled at the same time (2) it is more economical. Micro-NT is especially suitable for seroepidemiological study where lots of samples have to be handled in short period.

18 ANALYSIS OF THE ENVELOPE GLYCOPROTEIN OF DENGUE VIRUS

TSUTOMU TAKEGAMI[1], CHIYOKO SEKI[2] AND SUSUMU HOTTA[1]

Institute for Tropical Medicine[1] and Department of Serology[2], Kanazawa Medical University

Envelope (E) glycoprotein seems to play an important role in dengue virus infection, like all flaviviruses. To elucidate the biological function of E protein, we prepared monoclonal antibodies against dengue virus type 1, Mochizuki strain. From the data of enzyme-linked immunosorbent assay (ELISA) and hemagglutination inhibition (HI) assay, those monoclonal antibodies were classified into at least 3 groups, i.e. (1) high titre in ELISA and HI, (2) high titre in ELISA but not in HI and (3) high titre in HI but not in ELISA. Monoclonal antibodies in each group could precipitate E protein of dengue virus labeled with ^{35}S-methionine. On the other hand, we determined the nucleotide sequence containing COOH-terminus of dengue virus-E protein, using reverse transcriptase and synthetic primer. Amino acid sequence deduced at its

COOH-terminus indicates hydrophobicity. These data suggest that COOH-terminal region of E protein may be embedded into virus envelope and other part of E protein has several antigenic determinants containing hemagglutination site.

19 MONOCLONAL ANTIBODY-BASED REVERSED PASSIVE LATEX AGGLUTINATION FOR IDENTIFICATION OF *VIBRIO CHOLERAE* O1

YASUO YANAGASE[1], KAZUE AOYAMA[1], KO SHOJI[1], FUMIO GONDAIRA[2], JUNJI MATSUDA[2], MASARU HIRANO[2], JUNICHI SUGIYAMA[2], YUUJI TERADA[2] AND CATHERINE P. RAÑOA[3]

Department of Bacteriology, Hyougo College of Medicine[1], Denka Institute of Biological Science[2] and Republic of the Philippines Ministry of Health San Lazaro Hospital, Manila[3]

We successfully prepared monoclonal antibodies against somatic antigen fractions a, b and c of *V. cholerae* and have developed a latex agglutination test for identification of *V. cholerae* types.

Stool specimens from 308 patients suspected of cholera in the Philippines were tested by the monoclonal antibody-sensitized latex agglutination test. A small portion was directly streaked onto TCBS medium to detect the presence of *V. cholerae*. The result showed that sensitivity of the latex reagents was sufficient for detection of *V. cholerae* O1 in stools from cholera patients.

It was concluded that the specificity and sensitivity of the monoclonal antibody-sensitized latex agglutination test were higher than those of polyvalent antisera and that the monoclonal antibody-sensitized latex agglutination test method for the diagnosis of acute cholera is very useful.

20 CHARACTERIZATION OF *VIBRIO CHOLERAE* O1 RECENTLY ISOLATED IN BANGLADESH

NOBORU NAKASONE[1], MASAAKI IWANAGA[1] AND ROGER EECKLES[2]

Department of Bacteriology, School of Medicine, University of the Ryukyus[1] and International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh[2]

Ninety-one strains of *Vibrio choleare* 01, isolated in Bangladesh in January 1986, were examined for their biological behaviour and sensitivity to 6 antimicrobial agents. Biotyping of the 91 isolates indicated that 60 of them belong to the classical biotype and 31 to the El Tor biotype, as determined by cholerae phage IV and polymyxin B sensitivity test. Twenty-one El Tor strains out of 31 revealed beta-haemolysis on blood agar plate, but only 8 strains revealed complete haemolysis in broth (Feeley and Pittman's method). Serotyping indicated 79 Ogawa, 10 Inaba (one classical and 9 El Tor), and 2 Hikojima (one classical, and one El Tor). Phage

typing showed that all classical vibrios belonged to Mukerjee's type 1. El Tor vibrios were classified into 4 groups: one strain each in type 1 and 5, 19 in type 4, and 10 were untypable group. Prophage typing of El Tor vibrios resulted in 14 strains of Ubol type, 16 strains of cured Celebes type, and one strain of original Celebes. In classical vibrios, there was a high productivity of cholerae toxin as compared to those isolated before 1973. There was no resistant strain against chloramphenicol, streptomycin, tetracycline, minocycline, amoxicillin and nalidixic acid.

21 COMPARATIVE STUDY ON DRUG SUSCEPTIBILITY OF *AEROMONAS* SPECIES ISOLATED IN BANGLADESH AND NAGASAKI

KAZUNORI SHIMOGUCHI[1], CHIKAKO MOCHIDA[1], KEIZO YAMAGUCHI[1], NOBUCHIKA KUSANO[2], TATSURO NAITO[2], SIGERU KOHNO[3], ATSUSI SAITO[3], KOHEI HARA[3] AND K. M. S. AZIZ[4]

Department of Laboratory Medicine, Nagasaki University School of Medicine[1], Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University[2], The 2nd Department of Internal Medicine, Nagasaki University School of Medicine[3] and ICDDR, B Hospital, Bangladesh[4]

Drug susceptibility test was performed on the 134 strains of *Aeromonas* spesies to the 16 antimicrobial agents including ABPC, MPC, PIPC, CEZ, CEX, CCL, CTM, CZX, CP, GM, KM, TOB, TC, MINO, ST and NFLX. Minimal inhibitory concentration (MICs) were determined by the microbroth dilution technique by using MIC 2000 (Dynatech).

The 134 strains of *Aeromonas* species consisted of the 105 strains kindly provided by International Center for Diarrhea Disease Research, Bangladesh (ICDDR. B) and the 29 strains isolated from patients hospitalized in Nagasaki University Hospital. The drug susceptibility of the strains provided ICDDR. B was almost similar to that of the strains isolated from Nagasaki. Most of both strains was resistant to ABPC, CEZ, CEX and CCL, but susceptible to the other antimicrobial agents.

The 105 strains from ICDDR. B consisted of 29 *A. hydrophila,* 41 *A. caviae* and 35 *A. sobria* strains. The 29 strains from Nagasaki consisted of 14 *A. hydrophila,* 12 *A. caviae* and 3 *A. sobria* strains. Most of the strains of *A. hydrophila* and *A. caviae* showed resistance to multiple drugs such as ABPC, CEZ, CEX and CCL. On the other hand, about 50% of the strains of *A. sobria* were susceptible to ABPC and 10–30% of the strains were susceptible to CEZ, CEX and CCL.

As for the antibiotic resistant pattern of each species, nearly all the strains of *A. hydorphila* and *A. caviae* were resistant to ABPC and CEX concurrently, although the 40% of the strains of *A. sobria* were resistant to CEX only.

β-lactamase production was considered as one of the drug resistance mechanisms of *A. hydrophila* to both ABPC and CEX. Then, the substrate profiles of β-lactamase produced by *A. hydrophila* was examined by the micro-iodometric assay. The β-lactamase produced by *A. hydrophila* appeared to be penicillinase.

22 DRUG SENSITIVITY AND SEROTYPE OF *PSEUDOMONAS AERUGINOSA* ISOLTED FROM DRINKING WATER IN INDONESIA

YOSHIYUKI OKUWAKI[1], HISAE YANAI[1], KOICHIRO FUJITA[2], SETSUKO TSUKIDATE[2] AND MASATOSHI SUGIYAMA[3]

Department of Microbiology, Kagawa Nutrition College[1], Department of Medical Zoology, Nagasaki University School of Medicine[2] and Department of Hygiene, Juntendo University School of Medicine[3]

The present study reports the results of drug sensitivity and serotype of *Pseudomonas aeruginosa* isolated from drinking water in Indonesia in 1985 and 1986.

The number of isolated strains were 11 in 1985 and 19 in 1986.

Sensitivity testing to chemotherapeutic agents such as PCB, PIPC, MZPC, CER, CET, CFX, CMZ, CPZ, GM, KM, CP, TC, CL and NA were examined by disk agar diffusion method. Almost all strains were sensitive to PCB, PIPC, MZPC, GM, CP, TC, CL, NA and CPZ (the third generation cephem drug). Scarcely any sensitivity, however, was shown to the first generation cephem drugs (CER, CET) and the second generation cephem drugs (CFX, CMZ).

MIC values were measured in those strains which seemed to be resistant by disk agar diffusion method. Resistance of all strains to CER, CET, CFX and CMZ were found at very high concetrations exceeding 250 μg/ml or 1,000 μg/ml.

Serotypes of isolated strains were classified E type 6 among 11 strains in 1985. In 1986, however, serotypes were found in E type and F type 6 among 19 strains respectively.

23 EXAMINATION OF DRINKING WATER IN NORTHERN AFRICAN COUNTRIES

KIYOKO SAWADA AND HIROSHI SUNAGA

Mizuho Junior College, Nagoya

Bacteriological and chemical examinations were carried out of drinking water collected in Monrovia, Abidjan, Conakry, Alger, Tunis and Tripoli.

Free residual chlorine was detected only in less than a third of total tap water samples examined. Coliform bacilli were isolated from tap water in the 3 cities on the Atlantic coast and Tripoli where residual chlorine was less often found. Drinking water samples collected at restaurants in Monrovia were found frequently contaminated by coliform bacilli. Drinking water in private residences which had been passed through the filter was often contaminated, suggesting probable incrimination of inadequate use of the filter. On the other hand, table waters on the market were safe from contamination of the bacilli.

Of 21 strains of coliform bacilli isolated, *K. pneumoniae* was most frequently seen, followed by *Ent. cloacae, C. freundii, K. oxytoca* and also 2 strains of *E. coli.* MIC tests revealed that

there were found 2 resistant strains to SM and TC, respectively, and one to CP, though all strains were susceptible to pipemid acid.

Seven strains of enterococci were also isolated including 5 strains of *St. faecalis liquefaciens* and a strain of *St. faecalis* and *St. faecium,* respectively.

Total hardness and chloride ion concentration of tap water in Alger and Tripoli were in general higher than those in other cities. Especially in Tripoli almost all specimens exceeded 300 mg/*l* in hardness and 200 mg/*l* in chloride ion concentration. In some specimens the latter was over 1,000 mg/*l* and salty tap water was the obstacle in making tea in daily life.

24 WATER-STERILIZING CAPACITY OF AN EQUIPMENT MADE OF HOLLOW MULTI-POROUS FIBERS AND SILBER-ACTIVATED CARBON POWDERS

HIROSHI OHARA[1], ISAO EBISAWA[1], MAKIKO TAKAYANAGI[1], TOSHIHIKO KOMATSU[2] AND TERUKO KOHARA[2]

Department of Public Health, Toho University School of Medicine[1] and Department of Enterovirology, National Institute of Health[2]

The incidence of water borne infections is extremely high in the developing countries. In the face of the increasing number of the Japanese sojourning in such countries, the supply of the clear water is becoming more and more important; and any effective measures which assure the supply of clear water is desired. Recently a new type of water sterilizer made of hollow multi-porous fibers and silber-activated carbon powders was developed. In order to examine the efficacy of the equipment, the water sterilizing capacity was tested using various pathogens which were prevalent in the developing tropical countries.

Escherichia coli, Serratia marcescens, Salmonella typhi, Vibrio cholerae, Shigella sonnei and *Salmonella typhimurium* were used as the experimental bacteria. Sterilized water containing 10^8–10^9 of bacteria per one liter was filtrated with the sterilizer. The filtrate was passed through a millipore filter and the filter was cultivated on the Endo's medium. Besides direct filtration of the bacteria contained water, 2 other experiments were performed after pre-treatment of the sterilizer; i.e. a large amount of unclear river water or the tube water was passed through the filter.

As a result of 3 kinds of experiments carried on 6 kinds of bacteria, no viable bacteria was detected in the water obtained after filtration with the sterilizer. An experiment was also carried out using Rota virus, and no virus was detected after filtration.

The combination of hollow multi-porous fibers and silberactivated carbon powders was considered to be effective to eliminate bacteria and virus as well. The equipment, in combination with vaccination and precaution against taking raw foods, seems to be much effective in prevention against oral infections in the developing countries.

JAPANESE JOURNAL

OF

TROPICAL MEDICINE AND HYGIENE

Vol. 15 No. 2 **June, 1987**

CONTENTS

Published by

JAPANESE SOCIETY OF TROPICAL MEDICINE

c/o Institute of Tropical Medicine, Nagasaki University

12–4 Sakamoto-machi, Nagasaki 852, Japan