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A NEWLY RECOGNIZED PARATENIC HOST OF *PARAGONIMUS* SPP.

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Abstract: The possibility of albino rats as the hosts of 3 species of the lung-flukes is experimentally detected. Rats were each orally infected with 10 to 20 metacercariae of the bisexual type of *P. westermani*, *P. miyazakii* and *P. mexicanus* and were necropsied 60 to 150 days after infection. All of the worms of *P. westermani* and a part of *P. miyazakii* (21%) and *P. mexicanus* (74%) were found in the muscles of the rats. They were morphologically similar to excysted metacercariae and contained many excretory granules in their bladder. Worms of the youngest stage selected from them were fed to dogs and cat and 37.3-66.7% of worms were recovered from the animals after 103-202 days. These worms were parasitic within cysts in the lungs and most of them were fully mature. Thus, it was revealed that the bisexual type of *P. westermani*, *P. miyazakii* and *P. mexicanus* also take the rat as their paratenic hosts.

INTRODUCTION

It was revealed that the infection with the parthenogenetic type of *P. westermani* was caused by ingesting not only its metacercariae parasitic in fresh-water crabs and/or crayfish but immature worms living in muscles of the paratenic hosts (Miyazaki and Haba, 1976; Haba, 1978). Especially, the Japanese wild boar, *Sus scrofa leucomystax*, was a very important natural paratenic host of human paragonimiasis *westermani* in the southern part of Kyushu District (Miyazaki *et al.*, 1978a; Tokudome *et al.*, 1977). Other species of *Paragonimus*, however, were not investigated on their paratenic hosts, and the present study attempt to some species of *Paragonimus* on this problem.

MATERIALS AND METHODS

The used species of *Paragonimus* in the present study were the bisexual type of *P. westermani* (Kerbert, 1878), *P. miyazakii* Kamo *et al.*, 1961 and *P. mexicanus* Miyazaki and Ishii, 1968. The metacercariae of *P. westermani* obtained from a potamonid crab, *Geothelphusa dehaani*, collected at Nishiki-mura, Akita Prefecture, Japan. Those of *P. miyazakii* were obtained from *G. dehaani* collected at Rokuroshi, Iwakuni city, Yamaguchi Prefecture, Japan, and of *P. mexicanus*; from *Pseudothelphusa chilensis* collected at Tabacal in the Condebamba Valley, Department of Cajamaruca, Peru. From 10 to 20 metacercariae of those species were infected to female albino rats of the wistar strain weighing about 200 g (Table 1). They were

administered by oral infection to the experimental animals with water using an injection syringe with a slender vinyl tube. These rats were necropsied 60 to 150 days after infection (Table 1). Their visceral organs and cavities were examined for lung fluke infection and the worms were recovered. After being isolated from each animal, the lung, liver and muscle of the whole body were cut into slices 3 to 4 mm thick and kept in Ringer's solution at 37–38 C. Worms came out of them spontaneously and were collected in the solution after 6 to 8 hours. The juvenile worms recovered from the muscle of the rats were orally given to 3 dogs and a cat, which are known to be favorable hosts of those fluke, and these animals were necropsied 103 to 202 days postinfection (Table 2). The worms recovered were fixed in 70% alcohol, stained with carmine and mounted with permount. Morphological observations were made on these mounted specimens.

RESULTS

I. Developments and parasitic sites of *Paragonimus* in rat

The bisexual type of *P. westermani*

Five rats were each infected with 10 to 15 metacercariae and were necropsied 126 and 150 days after infection. All of the rats harbored 1–9 worms in the muscle. All of the 25 worms removed from the muscle were immature and were morphologically same as the metacercariae. Occasionally a small stylet was still visible in the oral sucker and excretory granules were seen in the bladder. No worms recovered from other parts of all of the animals except the muscle and no abnormal sign was found in their visceral organs.

P. miyazakii

Six rats were each infected with 20 metacercariae and were necropsied 100 days after infection. A summary of observations is shown in Table 1. A total of 57 worms (47.5%) was recovered and 20 of them were found from the cysts in the lung. Cysts were also formed in the livers (Nos. 2, 3, 4 and 5) and 11 worms were recovered from them and 2 worms were found to be migrating in the livers of both rats, Nos. 2 and 4. There was each one worm cyst in the renal adipose tissue and on the diaphragm and each contained 2 worms. Moreover, 6 worms were found in the cavities and hypoderm. From the muscle of these animals were recovered 12 worms. Fifteen worms in all from capsules in the liver, kidney and diaphragm and 6 from cysts in the lung and a worm from the pleural cavity were fully mature. Development of the remaining 17 worms from the lung and pleural cavity and 5 worms in the hypoderm, liver and abdominal cavity were fairly advanced, but were still young. The 12 worms in the muscle of 4 rats (Nos. 3, 4, 5 and 6) and 1 worm in the liver (No. 2) were very young and their morphology was similar to that of excysted metacercariae.

P. mexicanus

Five rats were each infected with 10 metacercariae and were necropsied 60 days

Table 1 Distribution of worms of the bisexual type of *P. westermani*, *P. miyazakii* and *P. mexicanus* in rats after infection with its metacercariae (Mc.)

Lung fluke	Rat No.	No. of Mc. given	Days of infection	No. of worms re-covered	No. of worms recovered from				
					muscle	abdominal cavity	pleural cavity	cyst in lung	others
<i>P. westermani</i>	1	10	126	9	9*	0	0	0	0
	2	10	126	8	8*	0	0	0	0
	3	10	150	4	4*	0	0	0	0
	4	10	150	3	3*	0	0	0	0
	5	15	150	1	1*	0	0	0	0
	N	55		25	25 (100%)				
<i>P. miyazakii</i>	1	20	100	9	0	0	2	7	0
	2	20	100	7	0	0	0	3	4 (1)*
	3	20	100	6	2*	0	0	2	2
	4	20	100	12	4*	0	1	0	7
	5	20	100	13	5*	0	0	2	6
	6	20	100	10	1*	1	1	6	1
	N	120		57	12 (21%)	1	4	20	20
<i>P. mexicanus</i>	1	10	60	6	6*	0	0	0	0
	2	10	60	4	2*	0	0	2	0
	3	10	60	5	5*	0	0	0	0
	4	10	60	4	1*	2*	0	1	0
	5	10	60	4	3*	0	0	1	0
	N	50		23	17 (74%)	2	0	4	0

* The worms are similar morphology to excysted Mc..

after infection. All of the rats harbored 1 to 6 worms in the muscle. All of the 17 worms removed from the muscle and 2 worms from abdominal cavity (No. 4) were very young and morphologically similar to excysted metacercariae. Occasionally a stylet was still visible in the oral sucker and pink granules were observed in the body parenchyma of the worms. One (Nos. 1, 4 and 5) or 2 (No. 2) worm cysts were found in the lung of these animals and they contained numerous eggs or 1 to 2 dead or almost dead worms.

II. Oral infection of dog and cat with juvenile flukes

The results of experimental infection of dog and cat with the different species of juvenile flukes which were collected from rat in the muscle are summarized in Table 2. Worm recovery rates of 2 dogs infected with *P. westermani* (bisexual type) were 53.3% (8/15) and 37.3% (3/8) which were examined at 103 and 202 days after infection, respectively. Ten worms from 5 worm cysts in the lungs were fully mature. A worm from the pleural cavity was less developed and without eggs in

the uterus. Worm recovery rate of a dog infected with *P. miyazakii* was 58.3% (7/12) when it was examined at 132 days postexposure. Six worms from 3 worm cysts in the lung were fully mature but a worm from the pleural cavity was less developed. Worm recovery rate of a cat infected with *P. mexicanus* was 66.7% (10/16) when it was examined 103 days postexposure. All of the 10 worms recovered from 5 worm cysts in the lung were fully mature. The prepatent period was 36 days after infection in the cat.

Table 2 Experimental infection to dogs or cat with immature worms of the bisexual type of *P. westermani*, *P. miyazakii* and *P. mexicanus* removed from rats

Lung fluke	Host animal	Days of infection	No. of worms	No. of worms recovered from		
			recovered/fed (%)	muscle	pleural cavity	cyst in lung
<i>P. westermani</i>	dog	103	8/15 (53.3)	0	0	8*
	dog	202	3/8 (37.3)	0	1	2*
<i>P. miyazakii</i>	dog	132	7/12 (58.3)	0	1	6*
<i>P. mexicanus</i>	cat	103	10/16 (66.7)	0	0	10*

* mature worms.

DISCUSSION

Paragonimiasis had been fairly prevalent in Japan but it is rare nowadays. Norimatsu *et al.* (1975) found 136 persons of pulmonary paragonimiasis, who were positive for the eggs of the parthenogenetic type of *P. westermani*, in Aira-gun, Kagoshima Prefecture. Most of the patients, however, did not eat fresh-water crabs and, moreover, the crabs distributed there (*Eriocheir japonicus* and *Geothelphusa dehaani*) were mostly negative for metacercariae of the lung fluke. They ate sliced raw flesh of wild boars. Miyazaki and Habe (1976) and Habe (1978) revealed by experiments that the lung fluke did not mature in the wild boar. Almost all worms were recovered from its muscle and were immature at 123 and 213 days post-infection. Most of these immature worms completely matured 64 and 80 days after feeding to dogs. These results suggested that the wild boar could be a new source of human infection with the lung fluke. This conclusion was supported by the results of an epidemiological study (Tokudome *et al.*, 1977). Miyazaki *et al.* (1978a) examined muscles of 3 wild boars captured in the endemic area and found immature *P. westermani* (parthenogenetic type) from 2 of them. It has been clear by experiments that the wild boar, pig, rat, mouse, hamster, guinea pig, rabbit, hen (Habe, 1978) and monkey (Miyazaki *et al.*, 1978b; Habe, 1982) could play a role as the paratenic host of this fluke. It has become clear also that the bisexual type of *P. westermani*, *P. miyazakii* and *P. mexicanus* take the rat as a paratenic host as the parthenogenetic type of *P. westermani* and sometimes the rat serves as the final host of *P. mexicanus* in the present studies. Such juvenile worm of *P. westermani* (bisexual type) as collected from the muscle of the rat in the present experiments was already

found from mouse, hamster, monkey, pig (Shibahara, 1981) and guinea pig (Habe, unpublished). The juvenile worm of *P. miyazakii* was collected from mouse (Yoshida, 1970), rabbit, hamster and guinea pig (Habe, 1979). Consequently, these species of *Paragonimus* have many paratenic hosts and this mode of infection may commonly take place among animals in nature. Man can be infected with some species of *Paragonimus* other than the parthenogenetic type of *P. westermani* by eating raw or undercooked meat of mammals which would serve as their paratenic hosts.

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パラテニックホストを持つ肺吸虫

波 部 重 久

ウェステルマン肺吸虫単為生殖型の終宿主への感染は、カニ類に寄生するメタセルカリアを経口的に摂取する他に、パラテニックホストになる哺乳類や鳥類を介しても成立する。特に南九州では、本虫のパラテニックホストであるイノシシの生食による患者が多発し、疫学上きわめて重要な問題となっている。そこで今回、他種肺吸虫について、このような感染経路があるかをみた。ラットにウェステルマン肺吸虫有性生殖型、宮崎肺吸虫およびメキシコ肺吸虫のメタセルカリアを10あるいは20個ずつ感染させ、60~150日の間に剖検した。ウェステルマン肺吸虫は回収虫体のすべてが筋肉から、メキシコ肺吸虫と宮崎肺吸虫は、回収虫体の74および21%が筋肉からであった。これらの虫体は、すべてメタセルカリアと類似した形態で、ほとんど発育していなかった。これらの幼若虫体を好適宿主であるイヌあるいはネコに経口投与したところ、その37.3~66.7%が感染し、肺に虫嚢を形成し、成熟した。この実験でウェステルマン肺吸虫有性生殖型、宮崎肺吸虫およびメキシコ肺吸虫が、パラテニックホストを介する感染経路を持つことが明らかになった。

ENZYME HISTOCHEMISTRY OF *BRUGIA PAHANGI*

1. Localization of Acid Phosphatase Activity in Developing Larvae in Mosquito (*Aedes aegypti*)

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Abstract: Histochemical localization of acid phosphatase was reported on the microfilariae isolated from the peripheral blood of the dog and their developing larvae in mosquitoes in comparison with the localization in those from the jird's peritoneal cavity and their larvae. Whole body of microfilariae stained red with dark red excretory vesicle and anal vesicle. The sheaths were negative in those from the dog, while positive in most microfilariae from the jird. During the first two days after the intake unstained larvae were frequently found in the mosquitoes fed jird microfilariae in contrast to those fed on the dog. From the third day on, positive developing intestine was observed. After the first molt the esophagus appeared as a red double line. On the 7th day strong activity was found at the esophagus, intestine, developing anus and amphids, while the nerve ring was negative. Positive reaction was detected at the hypodermis and anus of infective larvae.

INTRODUCTION

A considerable amount of research has been reported on the acid phosphatase activity in parasitic helminths. The enzyme is supposed to play an important role in the absorption of nutrients and excretion of metabolites by the parasites. Reports that two species of microfilariae which could hardly be differentiated from each other by the morphological characteristics (Aoki *et al.*, 1976) were easily classified by the different localization of acid phosphatase (Chalifoux and Hunt, 1971; Redington *et al.*, 1975) gave a new light on this enzyme from a stand point of taxonomic importance.

When the infective larvae of *Brugia pahangi* were inoculated into the peritoneal cavity of the jird (*Meriones unguiculatus*), adults and microfilariae were recovered from the peritoneal cavity (McCall *et al.*, 1973). The adults and microfilariae localized in the peritoneal cavity seem to follow an aberrant mode of development, although the microfilariae from the peritoneal cavity have been proved to reach stage III (*i.e.* infective stage) in mosquitoes as those from the peripheral blood of the dog which is a normal host of *B. pahangi* (Chuang *et al.*, 1979).

In this paper, a report is made on the localization of acid phosphatase in the developing stages of *B. pahangi* from the microfilariae in the canine peripheral blood

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to the infective larvae in mosquitoes. Also described is the localization of the same enzyme in *B. pahangi* microfilariae produced in the peritoneal cavity of the jird and their developing larval stages in mosquitoes in comparison with those from the dog.

MATERIALS AND METHODS

The microfilariae from the canine peripheral blood and those from the peritoneal cavity of the jird were smeared on coverslips. The mosquitoes used were Liverpool strain of *Aedes aegypti* maintained in our laboratory. A group of mosquitoes were fed on the infected dog with *B. pahangi* microfilaremia for about 15 minutes. Another group of mosquitoes were fed on the artificial feeding apparatus in which microfilariae from the peritoneal cavity of the jird were suspended in Dulbecco's phosphate-buffered saline without calcium or magnesium added with adenosine 5'triphosphate, 10^{-3} M (Chuang *et al.*, 1979). The mosquitoes from each group were then dissected in a drop of physiological saline on cover slips 2 hours and every 24 hours after the infecting feed. The preparations were then air-dried and stored at -20 C until the use. The stored preparations were used within 2 weeks with no recognizable decrease of the enzyme activity. Frozen sections of thoracic muscles of infected mosquitoes were made on 7th, 9th and 11th day after the infecting feed.

For the demonstration of acid phosphatase activity, the technique of Barka was used following the description by Chalifoux and Hunt (1971), in which naphthol AS-BI phosphate was a substrate and pararosanilin was a capturing agent. The pH of incubation medium was adjusted to 5.0. The time of incubation was from 60 to 90 minutes at 37 C. Localization of acid phosphatase was recognized as red to dark red precipitations of azo dye, depending on the strength of the reaction. Stained frozen sections were mounted according to the method of Wharton (1957).

Some preparations were processed without the substrate as a control. Sodium fluoride, 10^{-2} M was also used as a specific inhibitor of the enzyme.

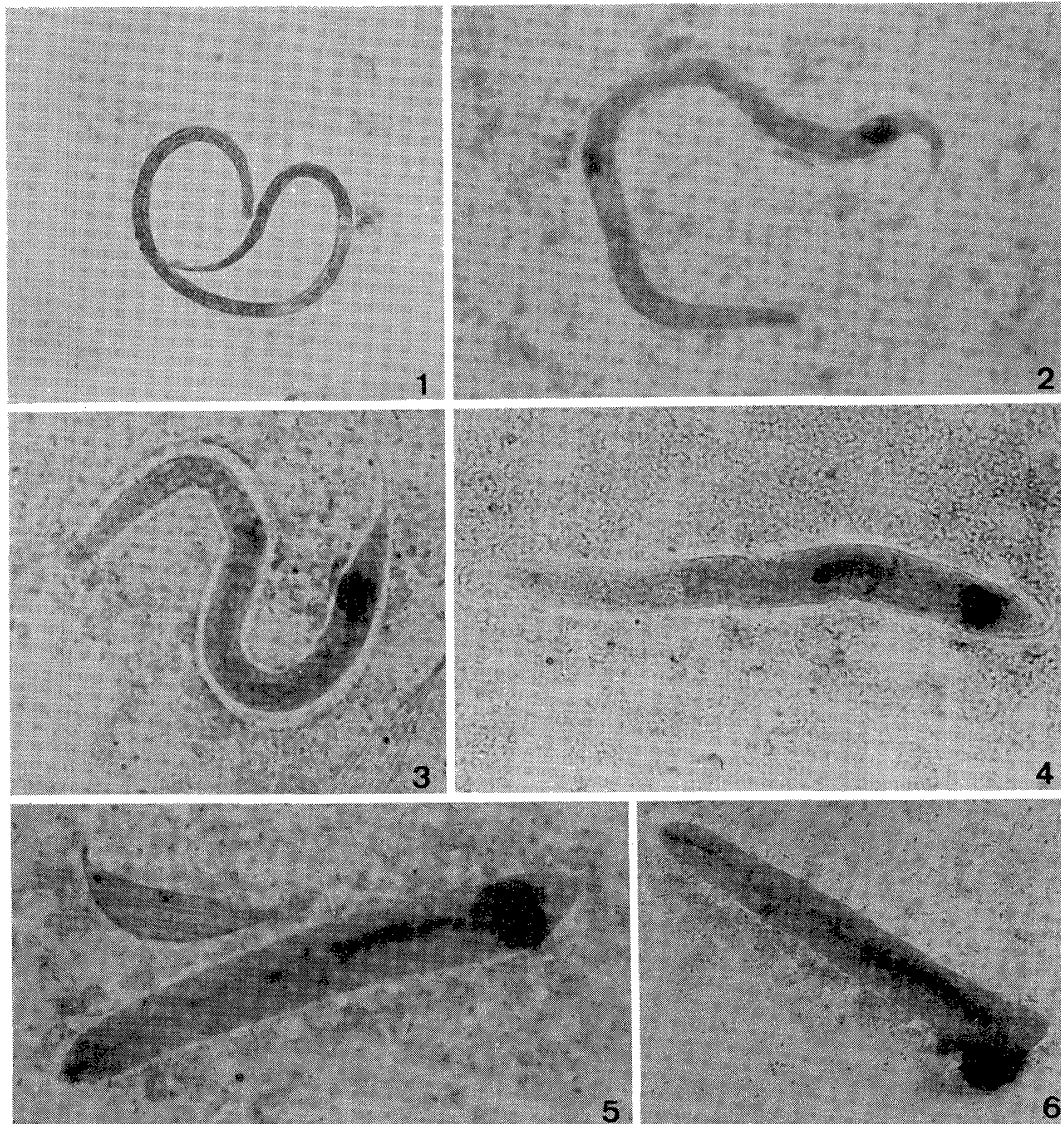
RESULTS

I. Direct Feeding on the Infected Dog

The whole body of microfilaria in the peripheral blood was stained red with two distinct dark red spots, excretory vesicle and anal vesicle (Figure 1). The inner body showed slightly weaker reaction in most of the microfilariae. With a few exceptions, microfilarial sheaths were negative for acid phosphatase. Occasionally the enzyme activity was recognized at the regions of amphids and phasmids.

Two hours after feeding, in the stomach of the mosquito, microfilariae showed the same staining pattern as in the peripheral blood.

Exsheathed first stage larvae were becoming shorter and thicker in the thoracic muscles of the mosquito 24 hours after feeding. The red excretory vesicle and anal vesicle were clearly seen in a light red tint of the larval body surface (Figure 2). On the second day, the strongest red reaction was found at the anal vesicle (Figure 3). In the light pink color of larval surface, the excretory vesicle became indistinguishable



- Figure 1 The microfilaria from dog peripheral blood stained red with dark red excretory vesicle and anal vesicle. Note the negative sheath.
- Figure 2 Body surface of exsheathed 1st stage larva stained light red. Excretory and anal vesicles showed strong reaction.
- Figure 3 Reaction of anal vesicle became stronger while that of excretory vesicle decreased on 2nd day.
- Figure 4 Anal vesicle stained deep red, while excretory vesicle was indistinct in 3rd day larva. Note the positive developing intestine.
- Figure 5 The positive intestine reached anal vesicle after 1st molt. Note the weak reaction at buccal cavity and esophagus.
- Figure 6 Vague red staining was sometimes found at excretory vesicle on 4th and 5th day.

in about 40% of the larvae.

The larva became shortest on the third day with increasing redness on the body surface. The anal vesicle was prominent with strong red color, but the excretory

vesicle was indistinct. Around at this time a dark red linear spot, which must be a developing intestine, first appeared in the region formerly occupied by the inner body and gradually grew backward to the anal vesicle (Figure 4).

During the 4th and the 5th day of development, when the first molt occurred, the red intestine reached the anal vesicle. Buccal cavity began to show weak activity of acid phosphatase and then esophagus appeared as a light red double line (Figure 5). In this stage, a vague red dot was found at the place of excretory vesicle in some larvae (Figure 6).

After the sixth day, when all larvae might be in the second stage, a daily rapid growth in larval size with strong phosphatase activity on the body surface began to disturb close observation of the internal structures in most of whole body preparations (Figures 7, 8).

Frozen sections of the 7th and 9th day larvae showed strong enzyme activity in the esophagus, intestinal cells and developing anus (Figure 9). The amphids were clearly positive (Figure 10), but nerve ring was negative. We failed to find the phasmids in the sections.

At the 10th day, whole body of third stage larva was deep red and the internal structures eluded observation, but ano-rectal area was still prominent with a strong enzyme activity, especially at the anterior wall of the rectum (Figure 11).

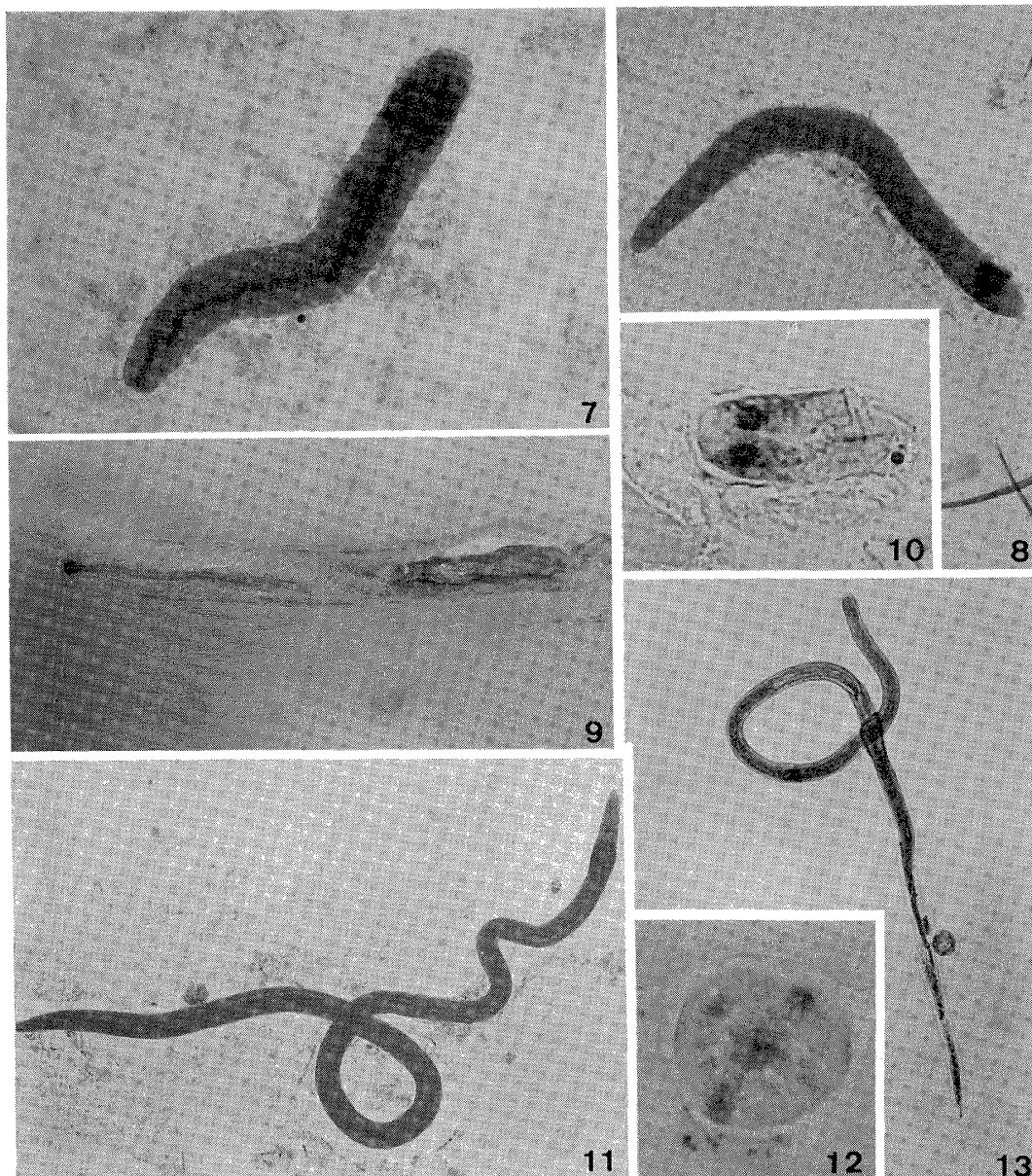
A mature infective larva (11 day old) had an open anus and whole body was evenly stained in deep red in whole body preparation. Frozen section of this stage showed positive enzyme reaction at main bulky parts of hypodermis which constitute lateral, dorsal and ventral cords, and very weak reaction in muscle tissues (Figure 12). Cuticle seemed to be negative, although it was not easy to distinguish cuticle and its underlying hypodermis under light-microscopical examination.

III. Artificial Feeding of Microfilariae from Peritoneal Cavity

The microfilariae from the peritoneal cavity of the jird were stained more clearly than those in the peripheral blood of the dog. The whole body except the inner body was pure red and the two vesicles were distinguished as dark red dots. Interestingly, most microfilarial sheaths (about 80%) were stained to variable extent from thin pink to strong red. Occasionally red small granules and/or red amorphous masses were observed in the space between the microfilaria and its sheath (Figure 13).

Acid phosphatase staining of the filarial larvae during the first two days after the infecting feed with artificial apparatus was unstable. Some larvae were stained fairly well but others were negative or poorly stained even at excretory and anal vesicles. After the second day, however, the enzyme activity of the larvae was found to be almost the same as in the direct feeding on the dog.

Control slides without substrate were all negative. Sodium fluoride (10^{-2} M) inhibited the reaction completely except a deep interior portion of anal region of third stage larva in whole body preparations. Most probably it resulted from a cuticular barrier against the penetration of the inhibitor.



- Figure 7 The positive esophagus and intestine were still visible in early 2nd stage larva.
- Figure 8 Internal structures except developing anus became invisible due to strong activity of body surface accompanied with rapid growth of 2nd stage larva in the whole body preparation.
- Figure 9 Note the strong reaction at amphids, esophagus and intestinal cells in frozen section of 7th day larva.
- Figure 10 Note the strongly positive amphids in frozen section of 9th day larva.
- Figure 11 Deep red stained whole body preparation of 10th day larva prevented observation of internal structures except strongly reacted ano-rectal area.
- Figure 12 Note the positive reaction at intestinal cells as well as lateral, ventral and dorsal cords in the cross section of infective larva.
- Figure 13 The microfilaria from jird peritoneal cavity stained well except the inner body. Note the positive granules in the space between the positive sheath and the body.

DISCUSSION

Many studies have been reported on the distribution of phosphatases and the functional significance in parasitic helminths. In earlier days, Rogers (1947) found alkaline phosphatase activity at intestinal cells of *Ascaris lumbricoides* and at cuticle of *Moniezia expansa*, and supposed that the enzyme might have relationship with carbohydrate absorption by the parasites. Erasmus (1957) showed the existence of both acid and alkaline phosphatases in *Taenia pisiformis* and related the enzymatic function to an active transport of materials. Robinson (1961) studied alkaline phosphatase of *Schistosoma mansoni* and speculated the possibility of carbohydrate absorption through cuticle. More recently, Parshad *et al.* (1977) showed the activities of phosphatases in excretory system of several different kinds of parasites. Stood *et al.* (1977) recognized acid phosphatase activity in the cuticle and hypodermis of *Haemonchus contortus* and considered that these are metabolically active sites. Maki and Yanagisawa (1979a) studied *Angiostrongylus cantonensis* electronmicroscopically and showed extra-lysosomal acid phosphatase which may suggest the transport of substances through the cuticle. Although there are few reports which show clearly the definite function of acid phosphatase in parasites, it is widely accepted that the enzyme plays an important role in absorbing or excreting substances through the mechanism of active transport. From this view, the excretory and anal vesicles must be main places of metabolism in *B. pahangi* microfilaria and the presence of acid phosphatase activity on the filarial body surface may suggest a transport of substance through the cuticle, although we could not clearly define the localization of the enzyme among the surface structures. Interestingly, Redington *et al.* (1975) reported that the body surface of *B. malayi* showed negative reaction for the enzyme.

In the early first stage, anal vesicle seems to be the most active place of metabolism, whereas the enzyme activity of excretory vesicle and body surface decreased rapidly. In the late first stage, partial development of buccal cavity, esophagus and intestine is observed (Laurence and Simpson, 1971), which will be followed by functional maturation on the 6th and the 7th day, although it may not be complete at this time. This is partially supported by the present findings and our observation that acetylcholinesterase activity appears in glandular region of esophagus from the 6th day of development (in preparation), and also by some electronmicroscopical studies by Buckett *et al.* (1970) and Tongu *et al.* (1978), who showed mitochondria of mosquito host in the larval intestine of *B. pahangi* on the 6th and 7th day, respectively.

In the third stage larvae, the esophago-intestinal system is the positive structure of the enzyme as in the earlier stages, indicating its absorptive function. Functional importance of enzyme activity in hypodermal cells has been discussed by many authors. It may work for transport of substance through the cuticle or production and maintenance of the cuticle (Dusanic, 1959; Sood, 1977). Recently Maki and Yanagisawa (1979b, 1980a, b, c) reported that gastro-intestinal nematodes showed high enzymic activity in the intestine and low in the body wall, and that tissue nematodes including filarial worms showed high activity in the body wall. They speculated that the body wall of tissue nematodes may play a role comparable to that of the

intestine of the gastro-intestinal nematodes.

Microfilariae from the peritoneal cavity of Mongolian jird showed a characteristic staining pattern of the phosphatase. It is very interesting that most of the filarial sheaths were stained to variable extent from pink to deep red, making a clear contrast to microfilariae from the peripheral blood of the dog. Acid phosphatase was also found in the space between the microfilaria and its sheath in a shape of small granules and/or amorphous masses. Omar (1977) studied the localization of acid phosphatase activity in the larval stages of *B. pahangi* in *Aedes togoi* and stressed the positive reaction of the enzyme in microfilarial sheath. But Redington *et al.* (1975) did not mention the acid phosphatase activity in the sheath of *B. pahangi*, in spite of the same technique as of Omar. Our present results made these conflicting reports more complicating, although the substrate used in our study was different from those of Redington and Omar. Our suggestion is that environmental factors may influence the metabolism of parasites and change the distribution or intensity of enzyme activity even in the same species. Moore and Halton (1976) reported a pronounced change in acid phosphatase distribution and its isozyme pattern between starved and nourished *Fasciola hepatica*. Edwards *et al.* (1971) and Pavlov *et al.* (1975) also reported the influence of host immunity on the parasitic acid phosphatase. At the same time, we have to keep in mind a fact that microfilariae in the peritoneal cavity are a mixture of young and old microfilariae (Chuang *et al.*, 1979). Aging, if any, may cause changes in staining pattern of the enzyme. In case of artificial feeding, many larvae were stained poorly during the first two days after feeding. The finding may also be related to the fact that the artificial feeding produces less infective larvae than the direct feeding (Chuang *et al.*, 1979).

Omar (1977) observed acid phosphatase activity at the phasmids and the nerve ring of *B. pahangi* larvae on the 6th and 7th day. Alkaline phosphatase was also found to be positive in the nerve ganglion of *Fasciola hepatica* (Humiczewska, 1975) and the nerve cord of *Ligula intestinalis* (Arme, 1966). However, we could not detect any positive reaction at the nerve ring, although the amphids and phasmids were positive in microfilarial stage and amphids were stained red on the 7th and 9th day larvae.

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Brugia pahangi の酵素組織化学

1. 蚊 (*Aedes aegypti*) 内発育幼虫における酸性フォスファターゼ活性の局在

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イヌ末梢血中ならびにスナネズミ腹腔中のマイクロフィラリアと、それらの蚊体内で発育中の幼虫について、酸性フォスファターゼの局在を組織化学的に比較した。マイクロフィラリアは全体が赤く染まり、excretory vesicle と anal vesicle が暗赤色を呈する。イヌのマイクロフィラリアでは鞘は陰性であるのに対し、スナネズミのものは大部分が陽性である。イヌを吸血した蚊とは対照的に、スナネズミのマイクロフィラリアを摂取した蚊では、最初の2日間に頻回に染色されない幼虫を認める。3日目以降、陽性の発育中の腸管を見る。1回目の脱皮後に、食道は赤色の複線として見える。7日目に、食道、腸管、発育中の肛門、amphid は強い活性を示すが、神経輪は陰性である。感染幼虫の角皮下層と肛門に陽性反応を認める。

THE EPIDEMIOLOGICAL SURVEY OF MALARIA IN ASAHAN DISTRICT, NORTH SUMATRA, INDONESIA

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Abstract: The blood and spleen examination for malaria was carried out in six villages (Desa) of Asahan district (Kabupaten), North Sumatra from February 1980 to March 1981. In general, parasite rate proved to be low (average 2.0%) in this area and both species of *Plasmodium vivax* and *P. falciparum* were detected. Besides this, interesting aspects of malaria endemic in this area were revealed. First, positive cases were found only in four villages bordering the sea, and, again, malaria endemic area was restricted to a few subvillages (Lorong) close to the coast in each village. Secondly the regular survey performed in Lorong I and II of Desa Perupuk, one of six villages, at intervals of a month showed that the active transmission of malaria took place during the dry season from January to July but the transmission became inactive during rainy season from August to December. The role of *Anopheles sundaicus*, which was determined as a main vector, in the limitation of endemic area and in the seasonal fluctuation of transmission was discussed.

INTRODUCTION

Since 1977, the malaria epidemiological studies have been conducted in Asahan district of the North Sumatra Province as one of the main activities in "The project for the promotion of health in North Sumatra", the international cooperation program between the republic of Indonesia and Japan.

The present survey was undertaken from February 1980 to March 1981 at three Kecamatan (subdistricts) in Kabupaten (district) Asahan to determine the current status and the epidemiological character of malaria. The entomological survey was also carried out by K. Tanaka and T. Ikemoto at the same place and the same time.

DESCRIPTION OF AREA

Six villages in three Kecamatan of Kabupaten Asahan were selected for the survey (Fig. 1). They are situated on a plain and approximately 200 km southeast of Medan, the capital of North Sumatra, and the population of each village varies from 2,000 to 6,000. Four villages border the Straits of Malacca and two villages are 5 to 15 km far from the coast. Inhabitants of the former engage in fishing and

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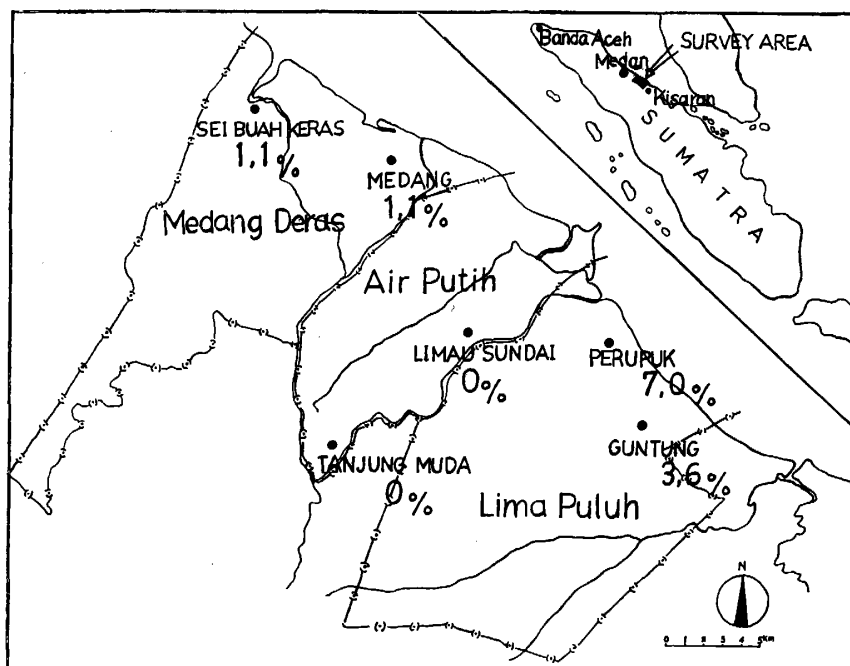


Figure 1 Map of six villages for the survey in Kabupaten Asahan, North Sumatra Province and results of blood examination for malaria parasite.

rice farming and inhabitants of the latter engage in rice farming. Rainfall patterns take maximum precipitation from September to November and minimum in January or February.

MATERIALS AND METHODS

I. General survey

1) Blood specimens were obtained from finger tips of children aged 0 to 7 years. Thick and thin smears were made onto respective microscope slides and dried in the air. Thick smears were hemolyzed in distilled water after six hours drying and stained with Giemsa on the next day. Thin smears were fixed in methanol and stained with Giemsa on the next day.

2) Spleen palpation was applied to the same samples. When the number of samples was not enough, it was extended to children aged 8 to 10 years.

II. Regular survey

At the Lorong (subvillage) I and II in Desa (village) Perupuk, one of six villages where higher parasite rate was discovered in the preliminary survey, the regular blood examination of children aged 0 to 7 years was carried out at intervals of one month from June 1980 to March 1981. Furthermore, parasite rate in other Lorong was examined at a proper time.

RESULTS

The results of blood and spleen examination in six villages were shown on Table 1 and Fig. 1. Parasite positive cases of malaria were found in four villages, namely Desa Perupuk, Desa Guntung, Desa Medang and Desa Sei Buah Keras, all of which were located along the coast, but no case was found in other two villages, Desa Limau Sundai and Desa Tanjung Muda which were located apart from the coast. *Plasmodium vivax* and *P. falciparum* were detected in three villages and only *P. vivax* was found in one village. Spleen rate also showed high in Desa Perupuk and Desa Guntung, where higher parasite rate was shown. The distribution of positive cases in Desa Medang and Desa Guntung were shown on Fig. 2 and Fig. 3, respectively. All positive cases of malaria parasites and spleen palpation were found only at the Lorong VIII facing the sea in Desa Medang, and mostly at the Lorong II also close to the sea in Desa Guntung. Comparative studies of parasite rate by Lorong in Desa Perupuk clearly showed the relation between malaria prevalence and distance from the sea (Fig. 4, Table 2). As the inhabitants mainly live along the main road, the distance from the sea to each Lorong is approximately indicated by the distance from the sea to the main road passing there. High malaria prevalence was found in Lorong I, II, III, IV, and V, low in Lorong VII and X and none in Lorong IX, XI and XII. Monthly fluctuation of parasite rate at the Lorong I and II showed that parasite rate rapidly increased from June and reached to a peak in August, and thereafter sharply declined until January (Fig. 5). The decline from August was more predominant in *P. falciparum* than in *P. vivax*. All the positive cases were ranged on a graph by their density of parasitemia and by the month when they were examined (Fig. 6) to differentiate new infections (acute cases) from old ones (chronic cases), based on the idea that new infection generally showed higher parasitemia

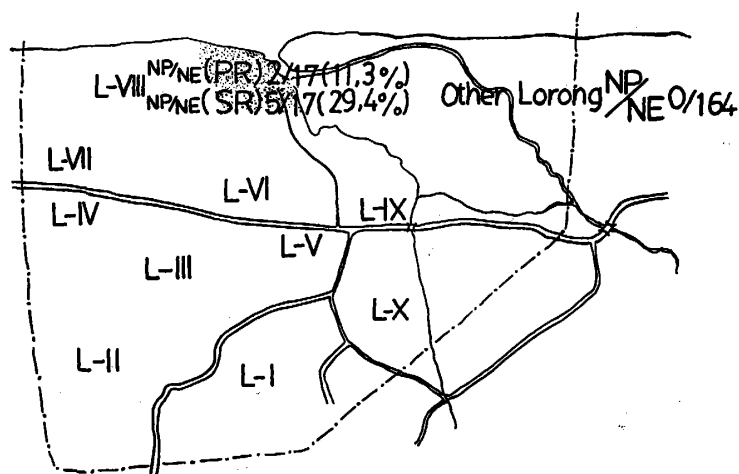


Figure 2 Map of Desa Medang and distribution of malaria positive cases. All positive cases were found in Lorong VIII facing the sea. NP: number of positive cases, NE: number of samples examined, PR: parasite rate, SR: spleen rate, L: Lorong.

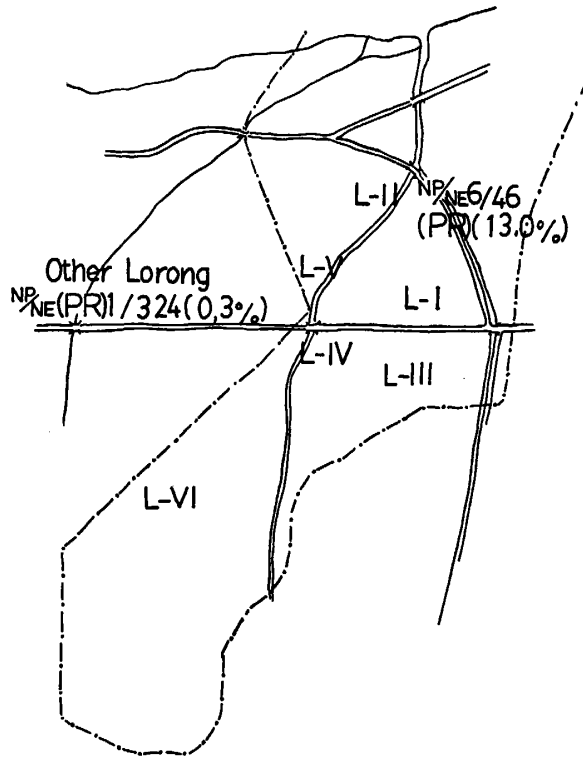


Figure 3 Map of Desa Guntung and distribution of malaria positive cases. Most of positive cases were found in Lorong II close to the coast. NP: number of positive cases, NE: number of samples examined, PR: parasite rate, L: Lorong.

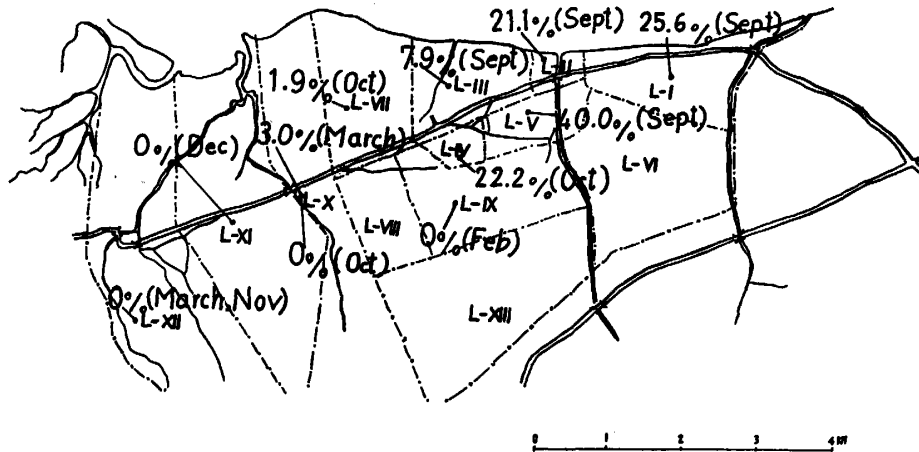


Figure 4 Map of Desa Perupuk. Comparison of parasite rate by Lorong. High parasite rate was found in some Lorong near the coast but none or low parasite rate was found in Lorong apart from the sea. L: Lorong

than old one. New infections were dominant from June to August while old infections were dominant from September to January. Again, new infections started increase from February.

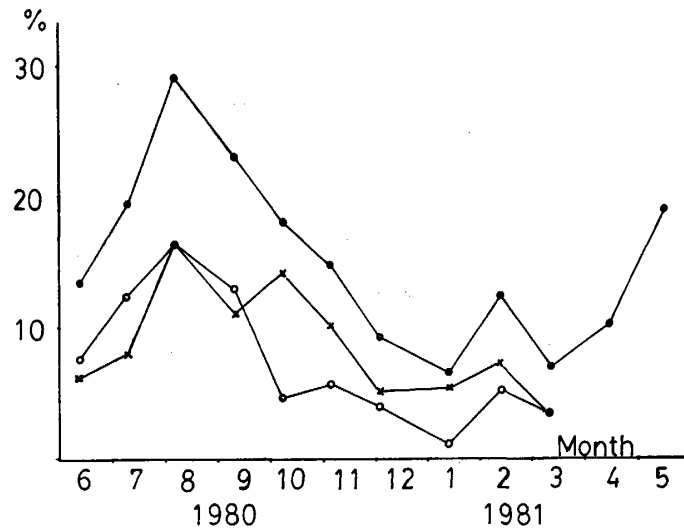


Figure 5 Monthly fluctuation of parasite rate in Lorong I and II of Desa Perupuk. Sharp rise from June to August and sharp decline from September to January was shown. Total parasite rate (●—●), *P. falciparum* (○—○), *P. vivax* (×—×). The data of parasite rate on April and May was gotten from Dr. H. Amano, the successor to the present reporter.

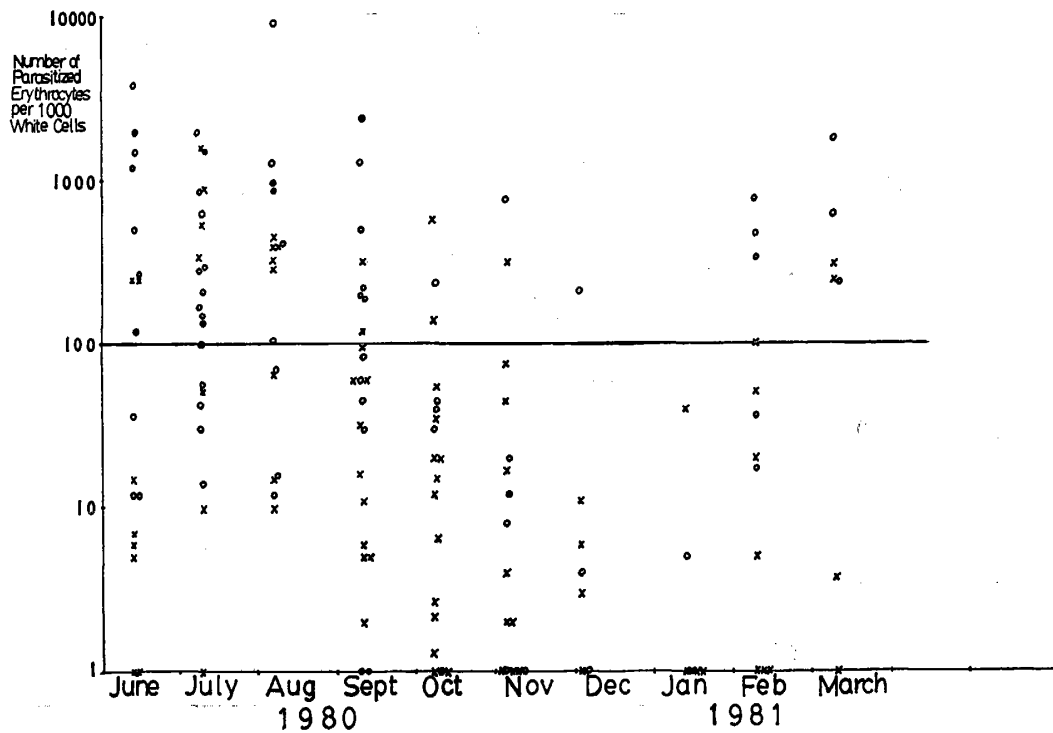


Figure 6 Ranging of positive cases of malaria by parasite density and by month when examined. *P. falciparum* (○), *P. vivax* (×), mix infection (●).

Table 1 The results of blood and spleen examination in six villages of Asahan district from February 1980 to November 1980

	No. examined	<i>P. vivax</i>	<i>P. falciparum</i>	Parasite Rate	Spleen Rate
Perupuk (Feb-March)	217	13	2	7.0%	5.1%
Guntung (March-Apr)	112	3	1	3.6%	10.7%
Medang (Apr-Jun)	181	2	0	1.1%	2.8%
Tanjung Muda (July-Aug)	209	0	0	0%	1.0%
Limau Sundai (Sept)	184	0	0	0%	0%
Sei Bua Keras (Nov)	279	2	1	1.1%	0.4%
Guntung (Oct)	370	2	5	1.9%	—
Total	1,552	22	9	2.0%	—

Table 2 Comparison of parasite rate by Lorong and by month in Desa Perupuk from February 1980 to March 1981

	Lorong Number											
	I	II	III	IV	V	VI	VII	IX	X	XI	XII	
Feb-Mar	5/19 (26.3)		1/43 (2.3)		6/36 (16.7)			0/15 (0)	2/67 (3.0)		0/28 (0)	
June	12/81 (14.8)	7/61 (11.4)										
July	9/64 (14.1)	13/49 (26.9)										
Aug	9/24 (37.5)	7/31 (22.4)										
Sept	11/43 (25.6)	12/57 (21.1)	5/63 (7.9)		14/35 (40.0)							
Oct	8/44 (18.2)	11/62 (17.7)		6/27 (22.2)			1/52 (1.9)					
Nov	6/55 (10.9)	10/53 (18.9)									0/60 (0)	
Dec	5/35 (14.3)	2/42 (4.8)			12/58 (20.7)					0/73 (0)		
Jan	0/34 (0)	6/59 (10.2)							0/84 (0)			
Feb	4/47 (8.5)	8/49 (16.3)										
Mar	3/48 (6.3)	3/39 (7.7)				3/32 (9.4)		0/27 (0)				

Each fraction shows number of positive cases/number of examined samples. A number in a parenthesis shows parasite rate (%).

DISCUSSION

Recently, the malaria control project was begun in Asahan district, North Sumatra by Indonesian Government, therefore the epidemiological condition of malaria in this area is clarified gradually. Up to date, some reports on malaria in the Sumatra island showed the absence of malaria in Bireuen and Takengon, Aceh Province (Stafford *et al.*, 1976), and very low malaria parasite rate in West Sumatra (1%) and in South Sumatra (2%) (Carney *et al.*, 1974, 1975). From these data, malaria endemic was expected to be low in Asahan district, North Sumatra, and our results confirmed this expectation in general. Besides this, some epidemiological characteristics became clear. First, the malaria endemic area proved to be distributed patchily along the coast and to be smaller than a village in extent. Tanaka and Ikemoto carried out the entomological survey in the same area and pointed out that *Anopheles sundaicus*, a suspected primary vector, was dominant among the man-biting anopheline mosquitoes in four villages where malaria positive cases were found while *A. sundaicus* was not discovered in two villages where no malaria cases were found. Furthermore, Ikemoto (1982) performed the comparative study of man-hour density of *A. sundaicus* by Lorong in Desa Perupuk. His result was well corresponding to our result of parasite rate by Lorong (Fig. 4). Accordingly we concluded that malaria endemic in the survey area was restricted to the narrow region close to the sea where *A. sundaicus* was able to breed. Secondly the malaria transmission proved to have seasonal fluctuation. It is known that the incubation period in human varies from 6 to 25 days for *P. falciparum* and from 8 to 27 days for *P. vivax* and that the extrinsic phase of *P. falciparum* requires 11 days and that of *P. vivax*, 8 days at temperature above 28 C. Considering these periods, the present parasite rate is thought to be the reflection of the malaria transmission around a month before. The parasite rate increased rapidly and new infections were dominant from June to August, whereas the parasite rate declined sharply and new infections were rare from September to January (Figs. 5, 6). New infections again appeared from February. These results meant that the active malaria transmission took place from May to July but declined from August. During the rainy season from August to December, the malaria transmission probably ceased but it recovered from January, the beginning of dry season, and became active thereafter; that was confirmed by the raised parasite rate in following April and May (Amano, unpublished data). The more rapid decline of parasite rate in *P. falciparum* than that in *P. vivax* is caused by its shorter clinical course. The seasonal fluctuation of parasite rate was also corresponding to the seasonal fluctuation of the density and longevity of *A. sundaicus* with nearly one month delay.

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インドネシア，北スマトラ州，アサハン地域におけるマラリア疫学調査

神原 廣 二¹・PANJAITAN, W.²

この報告はインドネシア共和国と日本政府による「北スマトラ地域保健プロジェクト」の一員としてマラリア疫学調査を行った結果をまとめたものである。調査は1980年2月より1981年3月まで北スマトラ州アサハン県の6カ村において行った。0-7歳までの乳幼児を対象として血液検査，脾触診を行った。また Perupuk 村においては，2つの部落を選んで6月より翌年3月まで毎月同じ集団よりの定期血液検査を行った。結果全体としてはマラリア寄生率2%と低いものであった。検出されたマラリアは熱帯熱と三日熱マラリアであり特に両者間の流行の差はない。ただこの調査を通じてこの地域に特徴的マラリア流行状況が明らかとなった。1つはこの地域のマラリア流行は海岸線に沿って存在し，しかも1つの村落内でも非常に海岸線に近接した小地域に限って行われている事である。もう1つは流行時期が乾期に限定される事である。寄生率の季節変動および個々の症例の原虫濃度による分類から，マラリア伝播は1月または2月頃より増加し始め，6-7月で最流行期を迎えるが，雨量の増加と共に減少して来る事が明らかとなった。この2つの特徴は池本，田中らによって調査された媒介蚊 *Anopheles sundaicus* の生態的特徴によって十分説明された。

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MICROFILARIAL DENSITY IN GUATEMALAN ONCHOCERCIASIS PATIENT'S SKIN WITH SPECIAL REFERENCE TO THE HOURLY INTAKE BY *SIMULIUM OCHRACEUM**

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Abstract: The present work was designed to assess the relationship between the hourly *O. volvulus* microfilarial density (mfd) in patient's skin and the hourly microfilarial intake by *S. ochraceum*. From two experiments employing 14 patients, it is suggested that there may be a little elevation of mfd during the period from evening through mid-night, but not pronounced. The microfilarial intake by the fly revealed two peaks, one in the morning and the other in the afternoon. Thus, the pattern of microfilarial intake did not correlate with the present diurnal pattern of mfd. The hourly intake was briefly discussed in relation to the biting activity and the parous rates of *S. ochraceum*. This report is the first case dealing with the hourly microfilarial intake by the black fly. Further such information would be required for a better understanding of the transmission mechanism of onchocerciasis in given endemic areas.

INTRODUCTION

The relationship between the microfilarial density in patient's skin and the hourly intake of *Onchocerca volvulus* microfilariae by *Simulium ochraceum* seems to have particular importance in the transmission of onchocerciasis in endemic areas. Recently, Campbell *et al.* (1980) working with Guatemalan onchocerciasis, stated that a nearly linear relationship was found between the mean skin mfd and the mean microfilarial uptake by *S. ochraceum* performing examination in the morning hours. Hitherto, there have been many works on the dermal microfilarial density (mfd) of onchocerciasis patients in connection with the biting activity of the vector, black fly, in endemic areas of the disease. In examinations at various times of the day, correlations between the peak mfd and biting activities of the vectors have been found in some geographical regions (Wegesa, 1966; Duke *et al.*, 1967; Lartigue, 1967; Thomas *et al.*, 1973; Duke & Moore, 1974; Anderson *et al.*, 1975). However, Picq and Jardel (1973), and Tada and Figueroa (1974) observed no diurnal periodicity in the mfd of *O. volvulus* in endemic areas of West Africa and Guatemala, respectively.

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Thus, the information on the diurnal periodicity of *O. volvulus* microfilariae in patient's skin still remains uncertain. In the light of transmission of the disease, however, it would be important to examine the intake of microfilariae by the vector, at various times of the day. Nevertheless, so far, little such an examination has been carried out. The microfilariae of *O. volvulus* may be attracted by the vector, black fly, when the flies are engorging on infected subjects (WHO, 1976; Hashiguchi *et al.*, 1981a); the number of microfilariae ingested may be very high in comparison with the densities observed in the human skin. It is assumed that the attracting condition, if any, may be influenced by times of the day in the given endemic area of onchocerciasis, since the black flies have a diurnal periodicity in their biting activity having two distinct peaks, one in the early morning and the other in the early afternoon (Collins *et al.*, 1981).

The present paper deals with hourly densities of the microfilariae in Guatemalan onchocerciasis patient's skin and hourly intake of *O. volvulus* microfilariae by *S. ochraceum*.

MATERIALS AND METHODS

The study area

The study was carried out from June 1978 through March 1979 in the following three coffee plantations with relatively high endemicity; *Finca* Santa Monica Ivone (Department of Suchitepequez), *Finca* San Rafael Sumatan and *Finca* Nimaya (Department of Chimaltenango) in Guatemala. They are located in the mountainous region 700 m to 800 m above sea level on the Pacific slopes of the Sierra Madre.

Skin biopsies on the subjects

Aiming at the assessment of microfilarial density in patient's skin, the skin biopsy was made at intervals of two to four hours, examining a total of 14 volunteers in the two study areas (*Finca* San Rafael Sumatan and *Finca* Nimaya). During night examinations, all the volunteers were permitted to sleep on bed, except the biopsy time. A Holth type sclerocorneal punch was used for the biopsy. Skin samples were taken from the left scapular region of the subjects approximately 0.5 cm away from the previous ones. The snips were incubated at 30 C in 0.85% NaCl for two hours and the numbers of microfilariae emerged were counted under a compound microscope at 40 \times magnification. The microfilarial density was expressed as the number per mm² of patient's skin.

Black fly collections

All the fly catches were made on the three infected adult male volunteers who wore the shirts with a 10 \times 10 cm grid at the back near the left scapula. The objective of the grid was to restrict the exposed skin for fly biting. In the three volunteers two skin snips each were taken from the exposed area; the mean rates obtained were used as mfd of the volunteers. The fly catches were performed from 0600 to 1800 of one day with hourly interval in *Finca* Santa Monica Ivone. All the

flies were captured after satiation with infected blood meal and then fixed immediately with 10% formalin and kept in the solution until dissection.

Dissection of flies

Since the migration of ingested microfilariae from stomach into other organs were prevented by above procedure, only abdominal parts of the fly were examined for the larvae. As the fixed flies with formalin had a coagulated blood meal in their stomach, they were treated in the laboratory as follows. The flies were washed with running water for two hours, incubated at 37 C in 0.5% KOH solution for 40 hours and then preserved in glycerin until examination. The abdominal region of flies was separated with insect needles under dissecting microscope, and then gently crushed with a slender teflon bar in a glass test tube with a drop of 0.85% NaCl solution. Care was taken to avoid any damage on the microfilariae. The materials were centrifuged at 1,000 rpm for 5 minutes; the supernatant was discarded, and Löffler's methylene blue solution was added to the sediments for the purpose of staining the microfilariae. All the samples were mounted on a slide with a coverslip. They were then examined under the microscope with 100× or 400× magnification. Since many microfilariae were destroyed by the cibarial armature of the fly during ingestion, the typical tail parts of the microfilariae were marked and counted, eliminating other fragments of the larvae. The microfilariae with only a small cut (less than about 10 μm) on the tail parts were counted as normal; thus very small fragments of the tail were also eliminated. The status of destruction in the present microfilariae ingested was similar to that found in our previous study in which the examination was carried out immediately after fly collections (Hashiguchi *et al.*, 1981b).

RESULTS

Microfilarial densities in the patient's skin

In order to assess the diurnal patterns of the skin density of *O. volvulus* micro-

Table 1 Microfilarial density in patient's skin, expressed as percentage per total mfd, in an endemic area, *Finca San Rafael Sumatan*, in Guatemala

Times	% mfd in each subject				Av. (%) ± s. d.	Gm**
	No. 1 (59.6*)	No. 2 (180.4)	No. 3 (94.8)	No. 4 (20.8)		
0900	13.1	9.9	7.2	6.7	9.2 ± 11.1	6.0
1200	24.2	12.0	7.3	2.9	11.6 ± 12.2	6.0
1500	5.2	17.6	6.8	20.7	12.6 ± 12.9	7.2
1800	10.6	22.1	7.0	9.6	12.3 ± 13.1	7.6
2100	8.6	16.5	15.0	14.9	13.8 ± 14.2	9.0
2400	19.1	5.5	19.1	9.6	13.3 ± 13.5	8.0
0400	11.7	10.1	12.8	26.9	15.4 ± 7.8	9.7
0600	7.6	6.3	25.0	8.7	11.9 ± 8.8	6.8

* Total mfd/mm²/day, ** Geometric mean.

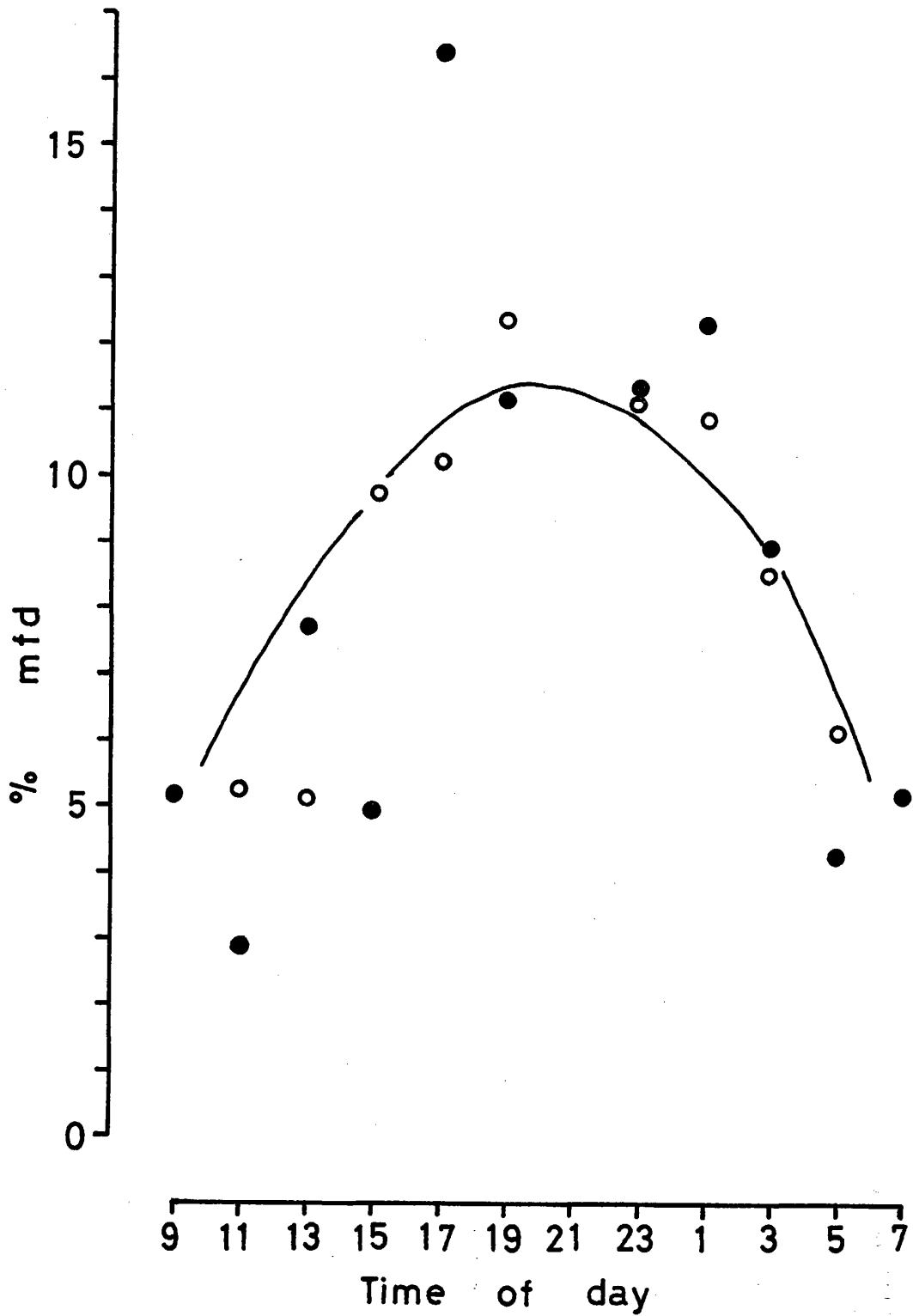


Figure 1 Microfilarial density of *O. volvulus* in 10 Guatemalan patient's skin, expressed as percentage per total mfd per day, in *Finca Nimaya*, Guatemala. ● mean % mfd; ○ triple-running average of mean % mfd.

filariae, quantitative skin biopsies were done at two endemic areas. In the first experiment, the biopsy was performed on four volunteers at intervals of two to four hours throughout the day, but the mfd varied with time and no clear-cut diurnal periodicity was recognized (Table 1). Table 2 and Fig. 1 give the results of second experiment by skin biopsies on 10 volunteers at the intervals of two hours in another endemic area. In this case, the mfd's tended to show a higher rate during the period from evening to mid-night, 1700–0100 (Fig. 1), though individual subjects revealed a considerable fluctuation by the time of day in their mfd's (Table 2). From the present two experiments, therefore, it is suggested that the mfd's in Guatemalan onchocerciasis patient's skin may have a slight elevation from evening through mid-night, but not pronounced.

Table 2 Microfilarial density in patient's skin, expressed as percentage per total mfd, in an endemic area, *Finca Nimaya*, in Guatemala

Times	% mfd in each subject										Av. (%) ±s. d.	Gm**
	No. 1 (37.5*)	No. 2 (29.8)	No. 3 (23.4)	No. 4 (87.8)	No. 5 (66.2)	No. 6 (9.2)	No. 7 (155.1)	No. 8 (101.1)	No. 9 (40.9)	No. 10 (108.0)		
0900	8.8	2.0	1.7	8.2	7.9	6.4	0.3	1.4	6.6	8.1	5.1± 3.4	1.8
1100	5.6	0.3	5.6	6.2	1.8	0.0	0.3	8.4	0.5	0.0	2.9± 3.2	0.7
1300	16.3	1.3	33.8	2.3	0.6	3.2	3.2	3.2	7.3	6.1	7.7±10.2	2.2
1500	1.6	1.0	11.1	3.1	6.2	4.3	5.0	6.0	0.5	9.7	4.9± 3.6	1.8
1700	14.9	12.1	0.0	13.2	22.2	14.9	47.0	17.7	8.8	13.9	16.5±12.2	5.7
1900	17.1	16.4	3.8	16.6	23.1	2.1	5.4	8.7	5.4	12.7	11.1± 7.0	4.6
2100	7.2	13.1	11.5	5.0	0.0	9.6	17.2	10.9	6.1	17.2	9.8± 5.4	3.3
2300	10.7	27.5	4.7	3.3	11.3	14.9	0.1	12.8	24.4	3.8	11.4± 9.1	3.3
0100	5.9	4.0	10.7	13.8	11.0	26.6	4.3	14.8	25.2	6.6	12.3± 8.1	5.1
0300	5.3	11.1	0.0	16.5	13.7	16.0	0.9	7.4	4.4	14.2	9.0± 6.2	2.9
0500	4.0	9.4	0.8	2.3	1.1	0.0	6.0	7.6	10.8	0.7	4.3± 3.9	1.4
0700	2.7	1.7	16.2	9.6	1.1	2.1	10.4	1.1	0.0	6.9	5.2± 5.4	1.5

* Total mfd/mm²/day, ** Geometric mean.

Hourly microfilarial intake by S. ochraceum

Table 3 and Fig. 2 show the hourly microfilarial intake by the fly, *S. ochraceum*, which fed each microfilaria carrier. A total of 360 flies, 132 from No. 1, 73 from No. 2 and 155 from No. 3 volunteers, were examined for the microfilariae, performing a mass dissection of three to six flies per batch. The total mean numbers of microfilariae per fly in each human bait, thus obtained, was 94.5 in No. 1, 20.4 in No. 2 and 42.6 in No. 3 throughout the day. The hourly intake of microfilariae by *S. ochraceum* had two peaks, one in the early morning, 0700–1000 and the other in the mid- or late afternoon, 1400–1600. To know the variation of microfilarial numbers ingested by a fly, a part of the samples collected from each patient during the morning hours (0700–0800) was dissected individually. The mean number of microfilariae per fly was 15.1±13.11 (n=15) in No. 1 (10.1 mfd), 3.5±7.43 (n=12) in No. 2

Table 3 *O. volvulus* microfilarial intake by *S. ochraceum*, expressed as percentage per total mean recovery of microfilariae from flies in each volunteer, at various times of the day

Times	Volunteer No. and (mfd/mm ² /skin*)						Av. (%) ± s. d.
	No. 1 (10.1 mfd)		No. 2 (13.9 mfd)		No. 3 (11.7 mfd)		
	No. fly exam.	% mf	No. fly exam.	% mf	No. fly exam.	% mf	
0600-	—	—	3	8.3	9	1.4	4.9±4.9
0700-	17	15.3	9	11.3	24	7.8	11.5±3.8
0800	13	9.2	11	14.2	20	18.3	13.9±4.6
0900-	8	3.3	4	1.5	19	8.0	4.3±3.4
1000-	12	4.8	12	11.3	18	13.1	9.7±4.4
1100	20	3.5	10	5.4	20	6.6	5.2±1.6
1200-	8	4.6	3	6.4	8	4.7	5.2±1.0
1300-	11	6.6	—	—	5	4.2	5.4±1.7
1400-	10	13.4	6	12.3	9	8.9	11.5±2.3
1500-	10	14.8	5	12.7	9	12.4	13.3±1.3
1600-	15	12.8	7	13.2	8	11.5	12.5±0.9
1700-	8	11.7	3	3.4	6	3.1	6.1±4.9

* Two skin snips each were taken from the volunteers at 15:30.

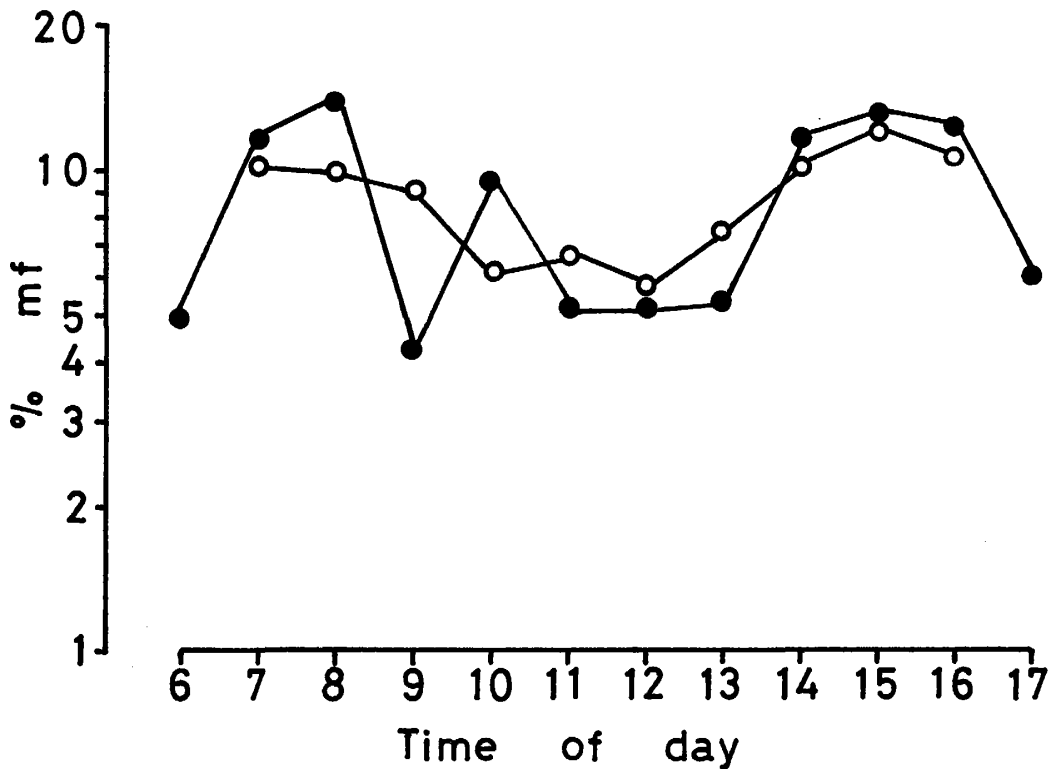


Figure 2 The intake of *O. volvulus* microfilariae by *S. ochraceum*, expressed as percentage per total recovery from flies in each human bait. ●—● mean % mf recovery; ○—○ triple-running average of mean % mf recovery.

(13.9 mfd) and 3.8 ± 4.32 ($n=15$) in No. 3 (11.7 mfd). Thus, the fly load indicated a great variation among the volunteers with a similar skin mfd. A similar observation on the low microfilarial intake by *S. ochraceum* was sometimes seen in the other field study.

Almost all of the microfilariae intaken by the fly were destroyed by the cibarial armature during ingestion; the damage reached 81.2% to 100% among those counted in the dissections of flies. Very low microfilarial intakes resulted in a high proportion of the destruction of microfilariae ingested.

DISCUSSION

It has been unknown whether the biting activity of black flies, *Simulium* spp., is influencing directly or indirectly on the *O. volvulus* microfilarial intakes. But there is a possibility that the microfilariae may be attracted by the vector, *S. ochraceum*, during ingestion (WHO, 1976; Hashiguchi *et al.*, 1981a). Therefore, the attracting condition of the black fly against skin microfilariae may be influenced by various factors, such as ecological and physiological ones, of the fly in various times of the day in endemic areas of the disease.

There were two peaks in the microfilarial intake, one in the early morning and the other in the late afternoon. However, this study is the first case reporting on the hourly intake of *O. volvulus* microfilariae by the vector, *S. ochraceum*. In order to have a more detailed and conclusive information, further such an examination should be done paying special attention to the parous ratios of *S. ochraceum* which showed distinct diurnal patterns (Collins *et al.*, 1981).

In the present study, the mfd of *O. volvulus* in 14 patient's skin was examined throughout the day in connection with microfilarial intakes by the fly, performing skin biopsies at 2 to 4 hour intervals. In patient's skin of one of the two experiments, the mfd rose slightly during the period from the late evening to mid-night indicating a considerable variation in individual volunteers. As to the mfd in skin snips taken at various times of the day, several studies are available in connection to the biting activity of the vector of Guatemalan onchocerciasis (Duke and Moore, 1974; Anderson *et al.*, 1975). Tada and Figueroa (1974), however, observed no diurnal periodicity in the skin microfilariae of *O. volvulus* in endemic areas of Guatemala. The discrepancy among investigators on the diurnal patterns of skin mfd may be due to the difference of sensitivities in skin biopsies. With the reason, it may be very difficult to know the true patterns of mfd in patient's skin by the biopsy techniques which are available in the moment. Collins *et al.* (1980), however, demonstrated that the sensitivity of the skin biopsy is enhanced by incubation for at least eight hours.

The result obtained suggests that there may be a diurnal pattern of microfilarial intakes of *O. volvulus* by *S. ochraceum*; the pattern, however, did not show any correlation with the present diurnal pattern of mfd in patient's skin. Further such an information on the hourly microfilarial intake would be necessary for a better understanding of the transmission mechanism of onchocerciasis. This study also showed that very low microfilarial intake resulted a high proportion of the destruction of microfilariae by the cibarial armature of *S. ochraceum*. The individual flies ingested such a low

density of microfilariae may be negligible in the light of their actual roles as a vector in the transmission of onchocerciasis.

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オンコセルカ症患者の仔虫密度とブユによる仔虫とり込み量の日周パターン¹

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グアテマラ共和国のオンコセルカ症流行地において、患者皮膚での仔虫密度とブユ *Simulium ochraceum* による仔虫とり込み量について、継時的観察を行った。患者4名について、2~4時間毎24時間、検皮法を実施した第1回目の実験では、仔虫密度に日周性を認めなかった。しかし、10名について2時間毎24時間の検皮では、午後5時から午前1時にかけて、仔虫密度の増加を認めた。一方、ブユにおける仔虫とり込み量は、午前と午後に高くなる傾向を示した。従って、今回の実験では、患者仔虫密度の日周性とブユによるとり込み量の間、相関を見出すことはできなかった。仔虫とり込み量の“2つの山”は、ブユの吸血活動に何らかの形で関与する可能性が示唆された。*Onchocerca volvulus* 仔虫のブユによるとり込み量についての継時的な観察は、従来、皆無である。しかし、オンコセルカ症の伝搬機構を解明していく上で、同様な研究が今後必要であると思われる。

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INCREASED T_{γ} CELLS IN PROTEIN CALORIE MALNUTRITION

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Abstract: A lowered T cell percentage and a raised T_{γ} cell percentage during the acutely ill period in Protein Calorie Malnutrition were observed. These values generally returned to normal after treatment. It is suggested that T cell immunodeficiency in Protein Calorie Malnutrition could be due to the alterations of T cell subsets coming from an increase of T_{γ} cells.

It is a well known fact that malnourished children have an increased susceptibility to infection. The interaction of malnutrition and infection results in a marked increase in morbidity and mortality among these malnourished children. Several investigators have shown atrophy of lymphatic tissue, impairment of delayed cutaneous hypersensitivity, suppression of phytohemagglutinin-induced DNA synthesis and decrease of T cell count. These suggest that there must be some immunodeficiency in malnutrition. To investigate immunodeficiency in malnutrition we examined the changes of T cells and T_{γ} cells at the acutely ill condition on admission and after a period of therapy and convalescence. This report deals with these results.

PATIENTS AND METHODS

Fifty-five young children aged 2 months to 5 years were diagnosed to be suffering from Protein Calorie Malnutrition (PCM) on the basis of clinical features (growth failure, loss of subcutaneous tissue, skin and hair changes), decreased weight for height and low serum protein. All patients were admitted at Princess Marie Louis Hospital in Accra, the capital of The Republic of Ghana. Twenty-five healthy infants aged 6 months to 5 years served as controls.

Heparinized peripheral blood was collected, diluted to two times with Phosphate Buffer Solution (PBS), and lymphocytes were isolated by Ficoll-Hypaque gradient centrifugation. Cells were washed twice with PBS and resuspended in PBS at a concentration of 2×10^6 cells per ml.

Into a small test tube 0.1 ml of the lymphocyte suspension was transferred and 1×10^7 Sheep Red Blood Cell (SRBC) in Fetal Calf Serum (FCS) were added to it. The mixture was centrifuged at $200 \times g$ for 5 min and allowed to stand overnight in

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ice water. Thereafter the cell pellets were resuspended by gentle agitation and examined for the proportion of cells forming rosettes with SRBC.

The determination of T_{γ} cells was in accordance with the double rosette method. Both the lymphocyte suspension and Chicken Red Blood Cell (CkRBC) coated with anti-CkRBC antibodies of IgG class were transferred into a small test tube, and the mixture was allowed to stand overnight in ice water. After removal of the supernatant, SRBC in FCS were added to the remainder. The mixture was centrifuged at $200 \times g$ for 5 min and was allowed to stand overnight in ice water. The cell pellets were resuspended by gentle agitation and examined for the proportion of the double rosette cells (T_{γ} cells) binding both the SRBC and the CkRBC coated with anti-CkRBC antibodies of IgG class.

RESULTS

T cell percentage in normal Ghanaian children was found to be $79.8 \pm 3.4\%$ (male: $79.9 \pm 3.8\%$, female: $79.8 \pm 1.6\%$) and T_{γ} cell percentage to be $2.2 \pm 0.8\%$ (male: $2.4 \pm 0.8\%$, female: $1.8 \pm 0.6\%$). No difference between the sexes was observed.

Three different types of PCM, such as marasmus, marasmic kwashiorkor and kwashiorkor, showed similar changes between the acutely ill condition on admission and after a period of therapy and convalescence. Namely, the low T cell percentage and the high T_{γ} cell percentage on admission generally returned to normal ranges after therapy (Figs. 1, 2).

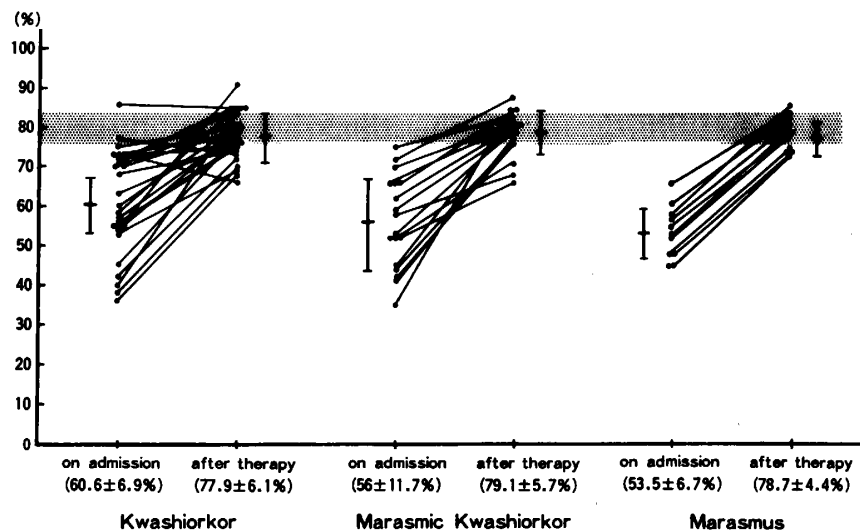


Figure 1 T cell percentages on admission and after therapy in kwashiorkor, marasmic kwashiorkor and marasmus.

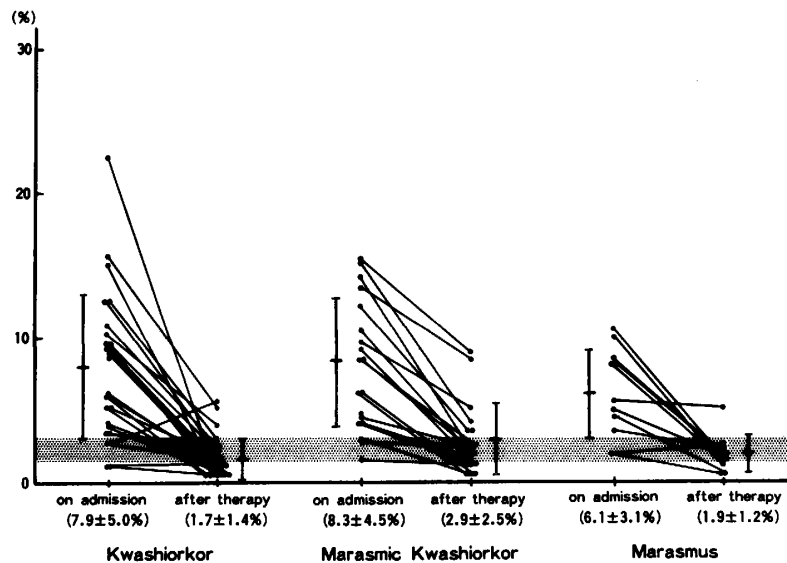


Figure 2 T_{γ} cell percentages on admission and after therapy in kwashiorkor, marasmic kwashiorkor and marasmus.

DISCUSSION

There have been no previous reports examining the T cell percentage and the T_{γ} cell percentage in Ghanaian children. Our T cell percentage of $79.8 \pm 3.4\%$ and T_{γ} cell percentage of $2.2 \pm 0.8\%$ are comparable with the values published for other national groups.

Concerning the immunological studies of PCM, there are some reports on the atrophy of lymphatic tissue, impaired delayed cutaneous hypersensitivity, the reduction of the *in vitro* lymphocyte transformation response to phytohemagglutinin stimulation and the decrease of T cell count (Smythe, *et al.*, 1971; Sellmeyer, *et al.*, 1972; Chandra, 1972; Schlesinger, 1974; Ferguson, 1974; Chandra, 1974; Neumann, 1975; Beatty, 1978). Moreover there is a report stating the normal B cell count and serum immunoglobulin value (Rafii, 1977). Looking through these reports it seems that there is T cell immunodeficiency in PCM.

We observed a lowered T cell percentage and a raised T_{γ} cell percentage during the acutely ill period in PCM. These values generally returned to normal after treatment.

PCM is divided into three types: Kwashiorkor which develops from marked deficiency of protein and a relatively excessive state of calorie marasmus which sets in at an absolutely deficient state of calorie, and marasmic kwashiorkor which is an intermediate type (Jelliffe, 1959). No significant difference was observed among them so far as the above changes of T cells and T_{γ} cells were concerned.

The T_{γ} cell is one of the T cell subsets, but functionally it is a heterogeneous population containing several subsets such as the suppressor T cell which suppresses the production of immunoglobulin from B cells and the killer T cell which possesses cytotoxicity.

It is suggested that T cell immunodeficiency in PCM could be due to the alterations of T cell subsets coming from an increase of T_γ cells.

Further investigation of the increasing T_γ cells in PCM from a functional aspect is expected.

ACKNOWLEDGEMENT

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蛋白質・カロリー欠乏症候群における T_H 細胞の増加について

関場慶博・小島滋恒・門井伸暁・田沼 悟・大原徳明

低栄養状態にある小児が感染症に罹患し易いのは、古くから良く知られた事実である。リンパ組織の萎縮、遅延型過敏反応の減弱、リンパ球幼若化反応の低下、T 細胞数の減少など、低栄養下での T 細胞性免疫不全を示唆する報告が多い。私たちは、この低栄養下での T 細胞性免疫不全にさらに検討を加える目的で、蛋白質・カロリー欠乏症候群での T 細胞、T_H 細胞の変動を、病盛期と回復期とで比較検討した。病盛期での T 細胞の低値、T_H 細胞の高値が、病態の回復とともに正常域へ回復して行くのが観察された。低栄養状態での T_H 細胞の増加という T 細胞 subsets の乱れが、T 細胞性免疫不全の一因をなしている可能性が示唆された。

日本熱帯医学会九州支部第6回大会講演要旨

期 日： 昭和57年1月23日（土）

会 場： 大分県医師会館大会議室

会 長： 大分医科大学微生物学講座 三舟求真人

（鹿児島大・医・熱研・熱帯病）

R. K. Anteson

（ガーナ大・野口研，医・微生物）

特 別 講 演

真菌症の病理

直江 史郎

（東邦大・医・大橋病院・病理）

- 7 ペルー産毒蛇 *Shushupe (Lachesis muta)* 及び *Jergon (Bothrops atrox)* の毒素性状について

Isabel Fukusaki

（サンマルコス大・ペルー）

後藤 勲，坂本 信一，山田 昭，
野中 実男 （化血研）

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(長崎大・熱帯医研・病理)

特別講演

真菌症の病理

直江 史郎 (東邦大・医・大橋病院・病理)

感染症は近年になり著しく変貌している。その基盤には抗生物質、免疫抑制剤、副腎皮質ホルモンが一般的に使用されるようになったことがあろう。すなわち、これらの薬剤の普遍化により以前は病原性の面からは重視されていなかった弱毒菌といわれているものの感染症が増加している。それらはしばしば *Opportunistic infection* として一括されることが多い。そのなかで真菌感染症は非常に大きな割合を占めているといえよう。

真菌感染症として臨床的に最も頻繁にみられるものはカンジダ症、白癬菌症であろう。症例が多くても臨床的にあまり問題がない癩風や外耳道真菌症なども存在する。一方、症例としても比較的多く、かつ臨床的には重要なものとしてアスペルギルス症、ムコール菌症、クリプトコッカス症、黒色菌症、放線菌症などがあげられる。しかし、臨床的には重要でありながらも本邦ではほとんど経験しない真菌があるが、これが熱帯地真菌症とそれに準ずるものが相当する。

以上のことから、本邦では基礎疾患がなく比較的感染力の強い真菌による原発性真菌症は非常に症例が少ないことから、病理学的観点から研究対象となるのはほとんどが続発性真菌症である。つまり、*Opportunistic fungus infection* のものが中心とならざるを得ない。すなわち、熱帯地に多くみられる原発性真菌症とは自ずから研究対象が異なっているといえよう。

病理学分野においてヒトを中心に真菌症の発生頻度をみる場合には主に日本病理剖検輯報を用いることが多い。それによれば、1958年から1977年の20年間に390,673例の剖検例が登録されており、そのうち真菌症は6,532例(1.67%)が記載されている。1958年の真菌症の発生頻度は0.59%であったのが、20年後の1977年には2.78%と非常に増加している。

剖検例からみると真菌症はカンジダ症(36.1%)が最も多い。続いてアスペルギルス症(26.0%)、クリプトコッカス症(10.1%)、ムコール菌症(3.6%)が多くみられるものの代表的な疾患である。

一方、熱帯地真菌症といっても、幾つかのものを除き熱帯地だけにみられるものはあまり多くない。熱帯地で発生頻度が高いというにすぎない。このような疾患には *Histoplasmosis*, *Coccidioidomycosis*, *North American blastomycosis*, *South American blastomycosis* (*Paracoccidioidomycosis*), *African histoplasmosis*, *Lobo's disease* (*Keloidal blastomycosis*), *Rhinosporidiosis*, *Mycetoma* などがあげられる。また、これらの疾患はしばしば地方病的様相を有していることが特徴といえよう。このうち、*Mycetoma* (*Madura foot*) を除くと本邦ではまず経験することはなく、もしこれらの症例に遭遇したとしても外国にて感染したものと断言しても良い位である。

真菌症の成立には個体の抵抗力の強弱が大きい。それと共に真菌が臓器に到達し、定着して増殖することが必要である。このとき菌量とその菌のもつ病原性が重要な因子として働くものと考えられる。

このような感染症を発生せしめる病原真菌は約100種類にすぎず、一般に真菌が5万とも10万種ともいわれていることから考えれば「医真菌学」は「真菌学」のなかではかなり狭いというか特殊な真菌を扱っているにすぎないといえよう。

しかし、真菌は病原真菌としてばかりでなく、毒キノコなど食中毒の原因ともなる。また、最近ではアレルギーとしての真菌が大きな問題となりつつある。さらには、真菌の代謝産物が生体傷害性を有したり、また発癌性(マイコトキシン)を産生するなどの有害な面がある。これに対し、薬

剤や醸造その他の利用面とを勘案するならば真菌はヒトの生活と密接な関連を有していることがわかる。このことから、医真菌学領域においてももっと広範囲な知識が必要となろう。それを示すかのように本年2月 New Zealand で行われる第8回 国際人獣医真菌学会のシンポジウムにて

“Mycotoxin and mycotoxicosis” のテーマがとりあげられることになり、少しずつ医真菌学の範囲が広くなりつつあるといえる。

今回は、真菌感染症の一般的事項を、熱帯地真菌症について触れながら、病理学的な面から述べた。

一般演題

1 急速に分布を拡大しつつある新しい衛生害虫ヒロヘリアオイラガについて

宮田 彬 (大分医大・生物)

ヒロヘリアオイラガ *Latoia lepida* (CRAMER) は、中国、インド、セイロン、ジャワ、スマトラ、スンダ列島に分布する蛾である。日本からは河田 (1930) の報告した、鹿児島県の“Toso” (福島英雄教授によると鹿児島市唐湊) 産の雌1頭だけが知られていた。ところが1960年になって竹村により、鹿児島市内で本種が再発見され、また1969年には鹿児島市内だけでなく、鹿屋市、佐多岬、屋久島などでも採集された。演者は1979年に大分医科大学に転動したが、ヒロヘリアオイラガが大分市およびその周辺の住宅地の街路樹に発生していることに気づいた。そこで同年秋の日本鱗翅学会総会 (福岡) で他の演題のついでに本種の幼虫および成虫のカラーズライドを供覧した。すると福岡県下の田主丸町や北九州市などでも発生しているという追加発言があった。その後さらに熊本市、大阪府下 (豊中市、池田市)、兵庫県下 (宝塚市)、静岡県下 (浜松市、湖西市、清水市)、岡山県下 (玉野市宇野) などでも発生が確認された。いずれも近年急に発生するようになってきたという。成虫の発生は年2回6-7月と8-9月で、後者に由来する幼虫はマユの中で前蛹状態で越冬するが、一部は蛹化して年内に羽化することがあるので、年3化もあるらしい。幼虫はナンキンハゼ、サルスベリ、サンゴジュ、カキノキ、クリ、ヒイラギモクセイ、ナツメ、アカメガシワ、セイヨウギズタ、サクラ、ウメ、モミジ、カナメなど種々の植物を食する。本種はもともと日本本土に土着していた種ではなく、1920年代に栽培植物の移入に伴ってインドあるいは中国から日本に入り、鹿児島市内で細々と世代を繰り返していたらしい。しかし近年の宅地造成に伴う街路樹や庭木の移動が原因で、急速に分布を拡大したらしく、今後さらに他の地点にも侵入する可能性が強

い。刺されると激痛があり赤くはれ上り、医師にかかった者も多いので、市街地や住宅地の衛生害虫として注意する必要がある。

2 グアテマラにおける *Onchocerca volvulus* の媒介可能ブユ種, *Simulium horacioi* の生態調査

高岡 宏行 (大分医大・医動物)

中米グアテマラにおいて *Onchocerca volvulus* の媒介可能種と報告された *Simulium horacioi* (*metallicum* グループ) の幼虫と成虫の生態を明らかにするため、オンコセルカ症浸淫地の3地区で周年調査を行った。*Simulium horacioi* の幼虫は、年間を通じて発生がみられ、特に年中恒常的に流れる小さな水系に多かった。また、雨期 (5-10月) にのみ出現する季節的水系にも発生することが観察された。人おとり法により捕集されたブユ雌個体群のなかで *S. horacioi* は、地区により異なるものの、0.06~29.0%を占め、*S. ochraceum* や *S. metallicum* s. str. とともに主要人吸血性ブユであることが判明した。この種の吸血活動には、9月ないし10月に極期となる明らかな季節変動が認められた。*Onchocerca volvulus* 幼虫の自然感染は *S. ochraceum* (第I, III期とも) および *S. metallicum* s. str. (第I期のみ) にみられたが、*S. horacioi* では743個体を解剖した限りではいずれの発育期の幼虫も見出せなかった。しかしながら、不明フィラリアの第III期幼虫、Mermithidの幼虫およびカビに感染されていた雌個体が少数みられた。今回の調査で明らかにされた知見を考慮して、今後のグアテマラにおけるオンコセルカ症の伝搬調査では、*S. ochraceum* や *S. metallicum* s. str. と同様、*S. horacioi* も検討の対象とする必要がある。

3 宮崎県における寄生虫感染調査成績

石井 明, 今井 淳一, 阿部 達也,
大橋 真, 下村 浩, 堀井洋一郎,
坂本 修, 常俊 義三

(宮崎医大・寄生虫・公衆衛生)

近年日本における寄生虫対策は腸管寄生線虫などに見事な成果を納めつつあるが、一方少ないとはいえ山間村落などに未だ感染があるので手をゆるめる事は出来ない。1978年以来、宮崎県農山村を主な対象として検査を実施した。鉤虫感染が特に山間村落にみられ、高い所で43%があったが、平均9.7%であった。高年齢層にやはり高い。ズビニ鉤虫が優位であった。鉤虫感染者にアンケート調査を行ったら、頭痛、肩こり、たちくらみ等を訴える者も多く、貧血といわれた者が女性で半数みられた。尿尿処理を自家で行う者が89%であった。多数の検便は手間のかかる熟練作業なので、皮内反応他によるスクリーニングについて検討した。感染幼虫抗原のタンパク濃度 $20 \mu\text{g}/\text{ml}$ で良い感度と特異性が得られた。膨疹は7mm、発赤は20mmを境界に陽性とした。対策が実施されたりして、ある程度に感染率が低い状態で有用である。ELISAを用いて検討したが、IgG抗体では特異性が不良で、IgE抗体の方が良かった。RASTも用いられる。治療後IgE抗体は下がる傾向にあった。回虫、鞭虫の感染は少数であったが、横川吸虫感染が特に河川流域地に多かった。高い所で21%、平均で9.4%であった。アユをセゴシで生食するためと思われる。肺吸虫VBS抗原による皮内反応は平均23%陽性で、高い所で40%があった。イノシシの生肉を食べる事は少なくなりつつある様子である。これら陽性者の寄生虫学的確定診断は仲々困難な課題として残されている。1980年2例の熱帯熱マラリア患者がアフリカ旅行帰国者に発生し、救急処置で救命した。糞線虫、アニサキス、蟯虫、眼虫、トリコモナス、トキソプラズマなどについては今回は対象として取上げられなかった。

4 大分県北部のサワガニから見出されたウェステルマン肺吸虫有性生殖型

波部 重久, 宮崎 一郎

(福岡大・医・寄生虫)

周防灘にそそぐ駅館川の支流、恵良川上流(大分県宇佐郡院内町)で採集したサワガニに、ウェステルマン肺吸虫有性生殖型の寄生を認めた。サワガニのエラ、および肝臓の検査でのメタセルカリア寄生率は、23%(23/100匹)~89.7%(26/29匹)で、溪谷で採集したものに高率に感染していた。筋肉を含め検査したカニ50匹の結果は、26匹にメタセルカリアがみられ、合計835個のメタセルカリアを回収した。陽性カニ1匹あたりの平均寄生メタセルカリア数は32個、最も多いものでは199個であった。メタセルカリアのカニ体内での分布は、回収メタセルカリア総数の86.5%が筋肉、次いで肝臓7.8%、エラ4.0%の順であった。メタセルカリアの大きさは92個の平均で $361 \times 351 \mu\text{m}$ ($296 \sim 412 \times 276 \sim 404 \mu\text{m}$)で、これまで本邦で報告されている同型のメタセルカリアの中で最も小さい。ラット、マウス、モルモットにメタセルカリアを感染させたところいずれも高率に感染したが、虫体は筋肉に侵入したまま発育を示さなかった。この未発育の虫体を回収し、イヌに与えたところ高率に感染し、成熟した。このことからウェステルマン肺吸虫の有性生殖型は、単為生殖型と同様 paratenic host を介する発育環をもつといえる。イヌおよびネコにメタセルカリアを感染させて得た成虫は、皮棘は単生、卵巣は多くのものが6葉に分れているが、100個体のうち16個体が4~5葉あるいは7~8葉に分岐する異常がみられた。精巣は卵巣側のものが6葉に、反対側のものが5葉に分れており、分葉異常を起こしているものは少なかった。虫卵は卵形で、大きさは100個平均で $79.4 \pm 5.6 \times 43.7 \pm 2.0 \mu\text{m}$ で、無蓋端に顕著な肥厚部を持つものが少数であるが、多くのものは肥厚部を持たないか、軽度のものであった。

5 ケニア国タベタ地区における住血吸虫症—マンソン住血吸虫とビルハルツ住血吸虫の混合感染について—

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住血吸虫症の感染防御免疫のうち, 特に交差免疫については, animal model による報告は多数あるが, 流行地における疫学調査に基づいた報告は少ない。

我々の教室では, 1974年以来ケニア国タベタ地区において, 住血吸虫症の調査を続けてきたが, 今回混合感染地区における159名の住民についての1974年の調査結果と, 1980年のそれとを比較し, 交差免疫の存在を示唆する結果を得た。それによれば,

1) マンソン住血吸虫卵だけ陽性のグループは, 両種の虫卵とも陰性だったグループに比べ, 6年後の再調査においてビルハルツ住血吸虫に対してかかりにくい傾向が見られた。

2) ビルハルツ住血吸虫卵だけ陽性のグループと, 両種の虫卵とも陰性だったグループとの間には, 6年後の再調査においてマンソン住血吸虫の感染に対しては差がなかった。

3) またタベタ地区では, 6年の間に治療が行われていたが, 外来治療による投薬では治療群と非治療群との間に差は見られず, マンソン住血吸虫卵だけ陽性のグループでは, むしろ治療を受けた方が, ビルハルツ住血吸虫に対してかかりやすくなっている傾向が見られた。

6 ガーナ国アクラおよびアクラ周辺における寄生虫感染状況

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西アフリカのガーナにおいては, 寄生虫の疫学的研究は殆ど行われていない。昭和55年6月より昭和56年12月まで, 同地において寄生虫の調査を

行ったので, 成績の一部について報告する。調査地は, Greater Accra Region の西部5部落およびアクラ市内の3病院, PML Hospital, Kotobabi Polyclinic, Ussher Clinic で, PML Hospital は小児専門病院, 他は Kotobabi, Ussher 地区の公立病院。検査法は, 各被検者より, 糞便, 尿, 血液塗抹標本を, 一部被検者からはこの他に静脈血を取り, 身長, 体重の測定を行った。糞便は, 直接塗抹, ホルマリン・エーテル法, 濾紙培養を併せ行い (試料収集の場所の関係上, 原虫検査には必ずしも好適な条件ではない), 尿は遠沈後鏡, 血液はギムザ染色を行った。Accra 市内の一般住民では, 被検者369例中, 寄生率49.6%, 回虫20.6%, 鉤虫20.0%, 大腸アメーバ13.5%, 糞線虫7.1%, ランブル鞭毛虫4.3%, 鞭虫2.4%, ビルハルツ住血吸虫2.4%, マンソン住血吸虫0.5%, 小形条虫0.5%, 腔トリコモナス0.3%, 広裂節頭条虫0.3%だった。このうち, 鉤虫は47例中46例がアメリカ鉤虫で1例がズビニ鉤虫であった。混合感染は, 2種混合感染38例 (10.3%), 3種混合感染9例 (2.4%)。農村部の就学前児童の寄生虫感染状況は, 寄生率51.8% (総被検者226例中117例) で, 回虫35.4%, ビルハルツ住血吸虫20.4%, 熱帯熱マラリア原虫9.3%, 鉤虫4.4%, 糞線虫3.5%, ランブル鞭毛虫1.8%, 四日熱マラリア原虫0.4%であった。下痢症の小児274例では, 寄生率35.4%で, 回虫20.8%, 熱帯熱マラリア原虫12.8%, 鞭虫3.6%, メニール鞭毛虫2.6%, 糞線虫2.6%, 鞭毛虫類2.2%, 大腸アメーバ1.1%, ランブル鞭毛虫1.1%, 鉤虫0.7%, 小形条虫0.7%, ビルハルツ住血吸虫0.7%であった。同一検体の細菌検査から, 次の病原細菌が認められた。Salmonella A, B, E₄, typhi, Shigella flexneri 4例, EPEC O 125: K 70, O 11: K 58, O 55: K 59, O 26: K 60。下痢と寄生虫との因果関係についても, 検討を行った。

7 ペルー産毒蛇 Shushupe (*Lachesis muta*) 及び Jergon (*Bothrops atrox*) の毒性性 状について

Isabel Fukusaki

(サンマルコス大・ペルー)

後藤 勲, 坂本 信一, 山田 昭,
野中 実男 (化血研)

ペルー産毒蛇 Shushupe (*L. muta*) 及び Jergon (*B. atrox*) の毒性性状を日本産毒蛇 Habu (*T. flavoviridis*) 及び Mamushi (*A. halys*) と比較検討したので、その成績を報告する。

1. 毒素量測定

各毒素の出血活性、致死活性、及び培養細胞に対する毒性に対して検討した。出血活性はウサギの背部皮内に希釈毒素を接種し、直径 10 mm の出血斑を生ずる毒素量を最小出血量 (MHD) として表わした。その結果、Shushupe 5.8 µg/MHD, Jergon 5.5 µg/MHD に対し、Habu 0.7 µg/MHD, Mamushi 0.1 µg/MHD であり、日本産毒蛇の毒素が強い毒性を示した。致死活性はマウス尾静脈内に希釈毒素を接種し、50%致死量 (LD₅₀) で表わした。Shushupe, Jergon, Habu の各毒素が 32 µg/LD₅₀ であるのに対し、Mamushi は 14.7 µg/LD₅₀ であった。

培養細胞に対する毒性は、5 種類 (VERO, HEL, FL, RK₁₃, BHK) の株化細胞に希釈毒素を接種し、50% Cytopathogenic Dose (CPD₅₀) で表わした。各毒素とも VERO と HEL では 0.32 µg/CPD₅₀ を示したのに対し、FL, RK₁₃, BHK では 3.20 µg/CPD₅₀ であり、VERO と HEL が他の細胞より 10 倍高い感受性を示した。

2. 抗毒素産生性

Shushupe, Jergon, Habu, Mamushi の各毒素でウサギを免疫し、その抗血清中の抗出血価、抗致死価、及び抗細胞毒性価について調べた。抗出血価 8~32 倍、抗致死価 4~16 倍、抗細胞毒性価は共に 64 倍であった。Shushupe 毒素でヒツジを免疫したところ、抗出血価 16 倍、抗致死価 8 倍、抗細胞毒性 128 倍の抗毒素を得た。

3. 各毒素とそれぞれの抗血清を用いて、交差寒

天ゲル内沈降反応を行った。その結果 Shushupe 及び Jergon 毒素と Habu, Mamushi 毒素との間に共通抗原の存在を推定させる沈降線を認めた。

4. 毒素成分の分析

Shushupe 毒素を DEAE-sepharose CL-6B によって分画した結果、6 つの蛋白のピークを得た。それぞれのピークを溶出順にピーク 1 から 6 と名づけると、致死活性はピーク 1 及び 2 に、出血活性はピーク 1, 2 及び 3, 4 に認められた。ピーク 5 及び 6 には毒素活性を認めなかった。各ピークをディスク電気泳動で調べてみると、各ピークになおいくつかの蛋白が認められるので、今後更に検討を進めたい。

8 ハブ毒による腎障害に関する研究

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尾辻 義人

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蛇毒の毒作用には神経毒作用、血液循環障害作用、溶血作用、出血作用、壊死作用および血液凝固阻止または促進作用などがある。実験的にはハブ毒を動物に投与することによって腎に特異的な変化—すなわちハブ毒投与初期には腎糸球体毛細血管の、のう胞状変化がみられ次第に増殖性変化へと変貌していくことが川路、坂口、森田、上田ら多くの研究者によって報告されている。今回私共は奄美大島を始めとする日本南西部諸島に生息するハブより得たハブ粗毒を Sephadex 75, 溶出液として 0.02 M, pH 7.2 のホウ酸緩衝液を用いてゲル濾過して得られた分画 FI, FIIa, FIIb, FIII をおのおの単独に体重当たり 0.5, 0.1 mg を雄家兎に投与して腎の変化を観察し以下の如き結果を得た。分画 FI 投与後 24 時間目に糸球体毛細血管の、のう胞状変化を認め、その内腔にフィブリン、赤血球、多核白血球を認め、次第にのう胞状変化は減少して、増殖性変化が増加、それと同時にフィブリンの析出はみられなくなった。こののう胞状腎に FITC 標識抗家兎 γ-グロブリン抗血清を用いての蛍光抗体法では全く蛍光はみられなかった。また電顕での electron dense deposit はみられなかった。分画 FIIa, FIIb, FIII 投与群ではのう胞

状変化も増殖性変化もみられなかった。また FI 投与による腎糸球体内で増殖した単核細胞の食食能をみるための墨粒を投与すると著明に墨粒が食食されているのが観察された。丸山によると分画 FI にはヒト血小板凝集惹起作用が, FIIa, FIIb, FIII にはヒト血小板凝集抑制作用があるといわれ, 今回の実験からハブ毒による腎障害発症には免疫学的機序より, むしろ血小板が何らかの関与をしていることが示唆された。

9 コレラ毒素の細胞内局在性

—免疫電顕及び蛍光抗体法を用いて—

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Ohtomo ら (1977), ついで Nichols ら (1980) は, コレラ菌毒素の subunit A (SA) が cytosol 分画に, subunit B (SB) が membrane 分画に局在することを報告している。本研究はその確認を目的として, 全菌体にいずれも間接法の酵素抗体法及び蛍光抗体法を適用したものである。

抗 SA 血清を用いた場合, 蛍光抗体法では菌体内に強く蛍光が認められ, periplasm から outer membrane にかけては観察されず, 酵素抗体法では DNA フィラメントが強度に electron lucent となって, 通常は DAB 反応の結果として osmiophilic となり electron dense な所見が得られるのとは逆ではあるが, periplasm から outer membrane には反応が認められなかったことと合わせて, 有意な所見と考える。抗 SB 血清を使用した場合は両法とも, periplasm から outer membrane に強い反応が認められ, cytoplasm には有意な所見はなかった。以上は前記先業を支持するものである。

10 ガス産生 *Shigella boydii* 14 型による輸入赤痢

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症例は34歳の男性で3日間のフィリピン旅行から帰国後殆ど毎日2~3回の泥状便が持続したが, 発熱や腹痛もなくアルコール過飲のためと思ひ放置していた。6カ月後長男(11歳)が, 発熱や膿粘血便などの典型的な赤痢様の症状を呈し, 便より赤痢菌を分離した。さらにその1カ月後に父親である本人が発熱や腹痛を呈し水様下痢便を認め入院した。便から長男と同じく *Sh. boydii* 14 型が分離された。入院時所見では左下腹部に抵抗があり圧痛を認める以外特記するような所見はなかった。入院時検査では白血球増加, CRP 3+, 便潜血反応3+と炎症所見がみられ, また下痢による脱水の傾向が伺われた。細菌学的検査では, 分離菌の生物学的性状はグルコースを分解, またガスの産生がみられインドールの産生はなく, ラクトースやマンニットを分解しないところからガス産生マンニット非分解の赤痢菌と考えられ血清学的に *Sh. boydii* 14 型と同定した。同居家族5人から分離された4株の薬剤感受性はテトラサイクリンとストレプトマイシン耐性であった。本例はガス産生 *Sh. boydii* 14 型による細菌性赤痢の本邦初の症例であるが, その生物学的性状が異なっていたため防疫的行政的には大腸菌として処理されており, 輸入感染症が増加してきている今日, 注意を喚起させられた症例であった。

11 中国の克山病の病因とその発病機序

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克山病 (Keshan disease) は1935年中国, 東北地方黒龍江省克山ではじめて報告された慢性の地方病性心筋疾患である。しかし調査がすすむに従って東北地方に限らず, 中国大陸の黒龍江省から西南部の雲南省にかけて, 幅狭い範囲で大陸を

縦断するように長く帯状の地域に発生していることが知られてきた。

はじめ冬期に多く発生し女子に多いことから一酸化炭素中毒説があったが、地域集積性があるため食物中の痕跡元素、特にセレン欠乏説、あるいは患者血液からコクサッキー B 群ウイルスを分離した報告があるためにウイルス説等がある。

患者は北部では冬に成人女性に多発し、南部では夏に小児に多発するという。このような疫学的知見から単一の病因と考えられないとも言えよう。今回、中国黒龍江省より克山病患者血清30例と対照正常人血清15例についてコクサッキー B 群ウイルスに対する中和抗体価を測定した。ただ、克山病患者血清はいずれも発病後6カ月より15年までの、しかも単一血清であったため中和抗体価から病原を推定することは極めて困難なことであった。

抗体価の算術平均値からみてコクサッキー B₂ 型と B₅ 型については検討の必要はないが、B₁ 型、B₃ 型、B₄ 型、B₆ 型については更に症例を増やし、特に B₄ 型については発病初期血清について検討すべきと考えられた。また、*in vitro* cell migration inhibition test を行い、ヒト心筋抽出液で高度免疫モルモットのマクロファージの遊走がコクサッキー B₄ 型ウイルスの添加によって阻止されることを知った。これは細胞性免疫の面からヒト心筋と B₄ 型ウイルスに共通抗原の存在を示すもので、反復した B₄ 型ウイルス感染は細胞性免疫という作用機序でヒト心筋を障害する可能性を示すものであろう。

12 狂犬病研究のためのマウス横紋筋肉腫細胞の樹立

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(長崎大・熱帯医研・病理)

森内 昭, 中山 巖

(大分医大・病理)

狂犬病ウイルスの筋肉内での増殖あるいは潜伏の基礎的研究の目的で、継代可能な筋肉細胞系の樹立を試みた。そこで 4-HAQO を用いて、ICR,

ddY, C₃H/He 系のマウスに、横紋筋肉腫を作らせ、その腫瘍細胞を *in vitro* で培養し、継代細胞系を樹立した。今回は、C₃H/He 系マウスから取り出した腫瘍細胞について検討を加えた。細胞は30代継代後クローニングして、5株の細胞クローンを分離した。得られた結果は次のようであった。

(1) 摘出した腫瘍組織について PTAH 染色、HE 染色、次に、継代したクローン細胞について、電子顕微鏡的観察、HE 染色、PAS 染色、PTAH 染色の結果、このクローンが filament に富んだ横紋筋肉細胞由来であることが判明した。

(2) クローン細胞の狂犬病ウイルスに対する感受性をみるため、ウイルス接種48時間後、螢光抗体染色した結果、細胞質内に多数のウイルス抗原が検出され、感受性であることがわかった。

(3) クローン細胞が myoblast から myotube への分化が可能かどうかについて検討した。培養液中への2% DMSO の添加、または 1.4 mM Ca⁺⁺ イオンを過剰に加えたり、減少したりすることによって、myoblast から myotube への分化を制御することが可能であった。この点でもクローン細胞が横紋筋肉細胞由来であることがわかった。

13 狂犬病ウイルスの細胞侵入機構

—ウイルス粒子の溶血能と細胞融合能の発現—

万年 和明, 大内 正信, 三舟求真人
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狂犬病ウイルスや VSV (牛水疱性口炎ウイルス) はヒト赤血球を凝集しないが、トリプシンまたはセミアルカリプロテアーゼで処理したヒト赤血球はこれらのウイルスでよく凝集されるようになり、酸性条件で溶血と著しい細胞融合が見られた。中性条件あるいは低温ではウイルスの溶血能および細胞融合能は見られなかった。一方、0.1 mM クロロキン存在下では狂犬病ウイルスおよび VSV の感染が阻害され、VSV の感染効率はクロロキンの存在で約1/100に低下した。以上の成績から狂犬病などのラブドウイルスの侵入機構

として、ウイルスはまず *Viropexis* で細胞内にとり込まれ、その水胞がライソゾームと融合して pH の低下が起こるやいなや、ウイルスエンベロープはライソゾーム膜と融合しウイルス遺伝子が細胞質内に侵入するというセムリキ森林ウイルスで提唱された機構と同様な事が考えられた。

14 温度適応に関する 2,3 の問題点について

小坂 光男, 土屋 勝彦, 大渡 伸,
井元 孝章

(長崎大・熱帯医研・疫学)

温度適応の形成過程にみられる生体側の温度感受性変化や温度刺激による生体側の調節反応の量的変化、さらに反応発現の様式に関する研究報告は多いが、これらを総合した温度適応の中核-末梢機序に関しては今日尚統一見解を欠いている。この原因としては、各研究者で温度順化の形成過程における反応・データの記録時点に差があり、報告結果に偏差が大きい事、記録される反応示標の数が少ない事、かつ、多種類の反応を同時記録した報告結果が乏しい事などが考えられる。

一般に、温度刺激によって誘発される①急性反応である神経性調節は比較的早期に慣れはやの現象をみるが、②続く体液性-内分泌性の慢性反応は比較的緩徐な馴れを生じ、③その時点において生体側には徐々に器質性変化が生じ、その増量と共に①②の反応は量的減少や反応発現閾値温度の変化が認められる。

例えば、発汗に関する実験報告では、皮膚の短期暑熱鍛練では発汗量の増加、季節的には夏期は冬期に比し発汗量の増加及び汗の電解質濃度 (Na^+) の低下、発汗開始の直腸温度は夏は冬に比し低温側に移動 (閾値変化) すると報告されている。しかし、熱帯地気候に長期生活しているフィリピン人は日本人に比し同じ暑熱刺激に対して発汗量が極めて少量であると報告されていて、所謂、*Habituation* (慣れ) の現象を示す。短期の暑熱

順化ラットの心拍変化は皮膚の温熱刺激に、一方、寒冷順化ラットの心拍数は冷刺激にそれぞれより高感度に反応する。同様に短期の暑熱-寒冷-順化ウサギの耳介及皮膚血管拡張-収縮反応は非順化対照群に比し増強されるが、逆に脳血流量は温度順化ウサギで減弱・減量がみられ、一見、慣れの現象を呈する。以上、温度順化の形成過程にみられる生体反応は測定時点において、量的かつ発現閾値に修飾がみられるので反応データ解析に問題を残している実状である。

15 高温環境下の運動中の発汗反応

佐々木 隆

(熊本大・体質医研・生理)

高温環境における作業中の発汗応答を連続的に観察し、体温との関連について検討を加えた。

4名の被験者 (成人男子, 28~30歳) を環境温度 30 C の室で30分間座位安静をとらせた後に、30 C あるいは 40 C (湿度45%) の人工気候室で自転車エルゴメーターによる運動負荷を行った。負荷の強度は 360 kgm/min で17分間を課した。発汗量はベッドバランスを用いて連続的に、また7カ所の皮膚温および直腸温はそれぞれ熱電対、サーミスターを用いて経時的に測定した。汗の蒸発を完全にするために、被験者の背部から送風を行った。

安静時の発汗パターンは 30 C と 40 C 環境で分時汗量は異なるが、ほぼ一定した変化を示した。これに対して作業時の場合は 30 C では13~14分、40 C では4~10分ごろを境として分時汗量の著明な増加による発汗パターンに変化が認められた。また 30 C では作業終了後3~4分で安静レベルに達するのに対して、40 C では10分以上を要する。平均皮膚温の低下は 40 C より 30 C において著しく、いずれの環境においても分時汗量の水準と皮膚温の低下度との間には有意な相関があった。

シンポジウム

ケニアにおける熱帯病研究の現状と展望

1 ケニア国伝染病対策プロジェクトとその
周辺の事情

林 薫

(長崎大・熱帯医研・ウイルス)

1976年8月、ケニア国から伝染病対策に関する協力要請がなされ、1977年、1978年の事前調査の後、1979年3月、プロジェクトが開始された。その目的はケニア国における主要伝染病に関する基礎調査と研究から得られる資料に基づいて疾病予防に関する基礎対策を具申することであり、期間は5カ年である。ウイルス、細菌、原虫寄生虫の各分野から、ケニア国内で広く問題視される下痢症の解析と住血吸虫症に関する疫学解明を主題として調査研究が行われている。現在まで派遣専門家は既に14名に達している。上記疾患についての調査対象は低地帯と高地帯とを選定し病因論的に比較対比することにし、今日まで両地帯の都市を中心に調査が行われてきた。現在では調査対象を村落集団へ移し、村落形態に伴う環境要因の分析、季節的変動などを検討し、流行要因の解析を行うことにしている。一方、わが国側の特別の配慮によってプロジェクト推進のため Communicable Diseases Research & Control Project Laboratory の建設が進められ1981年度で完成される。この研究棟では応用研究を行い、野外調査と平行して調査資料を検討してゆくことになっている。プロジェクトの進行過程にはケニア国研究者、技術者との協同作業を通じて技術移転を行うと共に質的向上を計り、他方、わが国の派遣研究者の学問的向上をも努めなければならないことは論を俟たない。

ケニア国における伝染病対策プロジェクトは以上のような方向性をもって既に各分野ともかなりの業績が集積されてきているが、その背景となるケニア国の地理的一般事情、医学研究態勢の推移、

国際機関の活動状況、医療行政と医学教育、などについて触れ、プロジェクト推進のためわが国の特に若手研究者の参加を期待し、その参考として諸事情について述べた。

2 細菌学の立場から

岩永 正明

(長崎大・熱帯医研・病原細菌)

昭和55年6月から56年8月の間、ケニア国伝染病研究対策プロジェクトに細菌学グループとして最初に参加し、主として細菌性下痢症に関する予備的調査を行った。当プロジェクトは5年計画であり、初期着任者は任期の大半を人間関係の設立、実験室の設営、研究活動をするための現地側との交渉、日本側チーム内での意見調整等に費さざるを得なかった。

細菌学グループの活動はコレラおよび一般下痢症の2つをテーマとして開始した。一般下痢症はニエリ・ナイロビ・モンバサの各州中央病院において、外来下痢患者からの病原菌分離を試みた。コレラに関しては、全国各地で分離されたコレラ菌の生物学的性状を調査すると同時に、血清型およびフェージ型からケニア国内におけるコレラの伝播ルートを推定した。結局、初年度に得た知見としては、1. 下痢性疾患の少なくとも50%以上は細菌感染によるものであろう。2. そのうち最も重大視すべきは細菌性赤痢である。3. ケニア国内におけるコレラの伝播はビクトリア湖周辺地区から中部高原都市へ、南部海岸地区から中部高原都市へ、またツルカナ地区(北西部)からの南下などが主たる経路であると考えられたことなどであった。

次に細菌感染症研究に関するケニアの現状を一言で述べると、それは極めて遅れているとしかいえない。細菌学者といえる者は全国でほんの1~2名しか居ない。臨床医も細菌感染症に対

する興味が少ないようで、特に下痢便が細菌検査に提出されることはむしろ稀であるともいえる。しかしたとえ臨床医が検査を依頼しても、そこにはもう一つの問題、即ち検査技術の問題をかかえていることが明らかであった。現状は全国的にみると単純な染色と鏡検によって判定出来るものまでといった段階であり、淋菌・髄膜炎菌はよく鏡検されている。今は分離・同定の検査が地方まで可能になる一步手前の状態であり、もう少しの努力で大きな進歩が期待される。

3 原虫学の立場から

井関 基弘 (阪市大・医・医動物)

ケニアで問題となる原虫性疾患としてはマラリア、トリパノソーマ症、リーシュマニア症(カラ・アザール)、赤痢アメーバ症、トキソプラズマ症などがあげられる。

マラリアは熱帯熱マラリアが95%以上を占め、四日熱マラリア、卵型マラリアが少数あり、三日熱マラリアは殆どみられない。媒介蚊は *A. gambiae* と *A. funestus* である。流行の主な地域は Victoria 湖周辺、海岸地方、Tana 川流域、それに Voi, Taveta 地方で、1980年 Victoria 湖北東にある Nandi 地方での調査結果では住民の40%以上、ある小学校では学童の50%以上が原虫陽性であったと報告されている。

トリパノソーマ症は Victoria 湖東岸の South Nyanza 地方に流行がみられ、*T. rhodesiense* によるもので、*Glossina pallidipes*, *G. fuscipes* が媒介する。1980年には6カ月間に61例の患者が検出されている。

カラ・アザールは中北部の乾燥地帯に流行がみられるが、Kitui の定着住民の間では内科的診断調査を行った19,255名のうち3,668名(19%)に感染が疑われたと報告されている。北部一帯では遊牧民の間に流行がある。*Phlebotomus martini* が媒介者であり、これはシロアリの巣(蟻塚)の通気孔内にひそんでいる。*L. tropica* による皮膚リーシュマニア症は西部のエルゴン山南側スロープに流行地がある。

赤痢アメーバは演者が滞在中に調査した6地域

の学童や一般住民2,977名の糞便のうち約30%からシストが検出された。

トキソプラズマについては Nyeri の州立病院の外来患者222名について血清調査を行ったが、55.9%の高率に抗体陽性が認められた。

さて、これら原虫性疾患に対するケニア側の研究の取り組みであるが、研究機関としては、ナイロビ大学医学部、ケニア医学研究所、ケニアトリパノソーマ症研究所、保健省疾病撲滅研究部、獣医学研究室、医学研究センター(ナイロビにあるオランダ王立熱帯病研究所がケニア側に移管されたもの)、臨床研究センター、それに国際研究機関として国際昆虫生理生態研究センターと国際動物病研究所などがある。それぞれの取り組んでいる研究内容については紙面の都合で省略するが、各研究施設単独あるいはいくつかかが協力して疫学、診断、治療、免疫学、生化学、生態学など各方面から調査・研究が進められている。これに米国の CDC や Walter Reed 陸軍(医学)研究所、オランダ熱帯医学研究所、英国、ソ連などの研究者が参加しての共同研究もみられる。

あらゆる疾病が豊富にあるという点でケニアは熱帯病研究の宝庫である。将来、日本としてケニアで研究を進めるには、これらケニア側の諸研究機関と緊密に連絡を取って、現地研究者との共同研究として、ケニア側の受ける利益を充分に考慮しつつ活動するよう方策を定めなければならないだろう。

4 寄生虫学の立場から

青木 克己

(長崎大・熱帯医研・寄生虫)

伝染病対策プロジェクト(JICA)における1981年5月以後の寄生虫班の活動内容を紹介した。

1. 寄生虫性下痢について

Coast Provincial General Hospital, Mombasa において、下痢を起こす病原体の検査が行われた。便の性状による検出される寄生虫種の違い等はみられず、寄生虫学的観察のみからは寄生虫性下痢は論じられない。腸管病原菌、ロタウイルスが検出されない下痢便には鞭虫、赤痢アメーバ、ラン

ブル鞭毛虫が高頻度に検出され、これらは寄生虫性下痢の重要な病原虫と考えられる。

2. 住血吸虫症の研究について

1981年8月より南部海岸沿いの Kwale 地区において住血吸虫症の研究がスタートした。本研究の Pilot area を設定すべく、南部海岸沿いの Kwale 地区における *Schistosoma haematobium* の分布を調査した。Kwale 地区の小学校135校より任意に41校を選び、その学校の4, 5, 6年生の検尿を行った。尿検査を受けた学童数は学校によって異なる(15~124)が、すべての学校に *S. haematobium* 虫卵陽性者が認められ(3~93%) Kwale 地区全域に *S. haematobium* が流行していることが明らかとなった。特に内陸の Kinango 地区では1校を除き、55~93%と高い虫卵陽性率がみられた。海岸沿いの学校の陽性率は一般に低い、80%を越える陽性率を示す学校も数校みられた。

1981年12月より行われる予定の本格的疫学調査の内容、実験室内で行われる各研究課題、本プロジェクトに関与するケニア国の共同研究者、プロジェクトを進めるにあたっての残された問題点等について報告した。

5 病理学の立場から

板倉 英世

(長崎大・熱帯研医・病理)

調査研究のいきさつと目的: 私共の研究室では

1971年に国際協力事業団の医療協力のもとに東アフリカ・ケニア共和国において病理解剖業務および臨床外科材料診断業務を開始したことから熱帯病の病理学的調査研究を始め現在に至っている。ここ数年間は文部省科学研究費もしくは特別事業費によって支援されている。学術調査の主題は、熱帯病の病理学全般について検討した結果、目下、肝炎・肝硬変・肝癌を主とした肝臓疾患の疫学と病理学、およびカポジ肉腫の疫学と病理学のこの2つの課題を選んでいる。もちろん感染症を主とした熱帯地域風土病について様々な面からの資料を集積している。これらの調査研究の最終目標は基礎的な情報を提供することによって治療と予防に貢献することである。また同時に、温熱環境と病態像の特異性との関連性を探るべく人体材料の基礎的資料の収集と今後の同方面の研究の見通しを確立したい。

現在行っている研究課題: 1. ケニアにおける B 型肝炎ウイルスの浸淫状況について血清学的調査研究。2. ケニアにおける肝硬変、肝細胞癌と B 型肝炎ウイルスとの関連性。3. ケニアにおける萎縮性肝硬変の組織像の特徴——特に炎症像の強さについて。4. ケニアにおける一般病理解剖や臨床外科材料に現われる主な肝臓疾患について。5. 東アフリカの食品に寄生するマイコトキシン産生真菌類の分離。6. アフリカ・バンツール語族の鉄沈着症。7. カポジ肉腫の疫学と病理組織発生。8. 熱帯環境と疾患の病態像。

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