LABORATORY MEETING

held under the auspices of the

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

on June 22, 1963 at the

PARASITOLOGY DEPARTMENT, UNIVERSITY OF SINGAPORE.

Dr. Don E. Eyles, Dr. A. A. Sandosham, Dr. M. Warren,
Dr. S. P. Ramakrishnan, Miss Elizabeth Guinn and Mr. Yap Loy Fong

A POSSIBLE NEW MONKEY MALARIA PARASITE FROM THE NILGIRIS IN SOUTHERN INDIA

Studies have been conducted of a parasite isolated from *Macaca radiata* in the Nilgiris, Madras State, Southern India (Ramakrishnan and Mohan, Bull. Nat. Soc. India, Med. Mosq. Dis., 9: 2, 1961). The characteristics differ from those of previously described parasites.

The periodicity appears to be tertian, but more studies need to be done on this important point. The ring stages are similar to those of *P. coatneyi* and also *P. knowlesi* and *P. falciparum*. The older trophozoites are extremely dense and compact and have the most abundant pigment seen by us in any monkey malaria parasite. The schizont appears to have about 15-19 merozoites. Distortion and a pinkish discoloration or vague Schuffner type stippling of the erythrocyte are produced. Maurer clefts are not seen. The gametocytes are round and have very heavy pigment, sometimes clumped in a few large masses.

The older asexual stages are quite abundant in the peripheral blood, but there does appear to be a distinct tendency for the parasites to recede from the peripheral blood as in *P. coatneyi* and *P. knowlesi* (particularly the new subspecies of Garnham). The closest affinities would appear to be with these two parasites.

Studies of the invasion of reticulocytes show that this parasite does not prefer the young erythrocytes. In contrast, the young trophozoites of *P. coatneyi* show a marked predilection for the reticulocytes. Should the periodicity be demonstrated with certainty to be tertian, there would appear little doubt that the parasite is undescribed. Should it later prove to be quotidian (which we believe is very unlikely) comparison must be made with the subspecies of *P. knowlesi* (the description of this subspecies is not in press, so its name cannot be used at this time).

Microscopical preparations showing the various erythrocytic stages were demonstrated.

Mr. Yap Loy Fong and Dr. A. A. Sandosham

BABESIA DECUMANI (MACFIE) AND OTHER BLOOD PARASITES IN A LITTLE SPINY-RAT, *RATTUS MUSSCHENBROEKI* (=*R. WHITEHEADI*)

Blood parasites identified as *Babesia decumani* (Macfie) by Marshall Laird (personal communication) were found in three giant long-tailed rats (*Rattus sabanus*) collected at the National Park in north Pahang by the Institute for Medical Research in 1955; six years later two further rats (*R. sabanus* and *R. bowersi*) from the same area were reported harbouring *Babesia* parasites presumably of the same species. According to the records kept at the Malaria and Filariasis Research Division of this Institute (see Table below) there was an infected *R. sabanus* in 1957 from the Ampang Reservoir Forest Reserve in Selangor.

The present finding of *B. decumani* in a *Rattus musschenbroeki* (=*R. whiteheadi*) from Batang Berjuntai, Selangor indicates another new host and a different locality.

FOREST RATS FOUND INFECTED WITH *BABESIA DECUMANI* (MACFIE).

Species (No. infected)	Locality	Year
Rattus sabanus (3)	National Park, Pahang	1955
" " (1)	Ampang Reservoir Forest Reserve, Selangor	1957
" " (1)	National Park, Pahang	1961
Rattus bowersi (1)	" " "	"
Rattus musschenbroeki (=*R. whiteheadi*) (1)	Batang Berjuntai, Selangor	1963

It is interesting to note that all the rats found with natural *Babesia* infections hitherto in Malaya belong to the forest species.

The Giemsa-stained thin blood film showed the parasites of *B. decumani* mostly as vacuolated ring-shaped unpigmented bodies which are not unlike *Plasmodium falciparum*, measuring 2-4 μ in diameter, and a few amoeboid forms with red nuclear chromatin and scanty pale blue cytoplasm in unenlarged erythrocytes.

Basic stained bacilliform organisms, 7-24 of them measuring about 1-1.7 μ in length and 0.3 μ in width were seen in a few erythrocytes. They resemble *Grahamella microti* Lavier.

Also found in the same blood film was a *Trypanosoma* sp. belonging to the *lewisi* group as classified by Hoare (1949). It is about 35 μ long with a maximum breadth of 7 μ. The posterior two-thirds of the cytoplasm stains an intense blue. The nucleus is large (2.3 μ) and rounded and is situated at about the middle of the body. The kinetoplast is about 1.5 μ behind the nucleus and the flagellum extends along the broad undulating membrane and beyond the anterior end the free portion measuring about 7 μ.

The thin film also showed unidentified microfilariae measuring about 118-163 μ long and 2-3 μ broad. The purple-stained nuclei are large and closely packed. The tail ends in a large rounded nucleus which gives it a clubbed appearance.

The blood film was demonstrated together with drawings made from the microscope of these blood parasites.

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Dr. Gordon F. Bennett, Dr. Don E. Eyles, Dr. McWilson Warren and W. H. Cheong

PLASMODIUM JUXTANUCLEARE (?), A NEWLY DISCOVERED PARASITE OF DOMESTIC FOWL IN MALAYA

Plasmodium juxtanucleare was first described by Versiani and Gomes in 1941 from galliforms in Brazil. Recently, Dhanapala (1962) described this parasite from galliforms in Ceylon. In a recent survey by this laboratory of culicine mosquitoes of a coastal mangrove region near Kuala Lumpur, sporozoites from a naturally infected *Culex sitiens* produced a *Plasmodium* infection when injected into a domestic chick. Subsequently this parasite was transmitted by (i) blood inoculation and (ii) sporozoites from experimentally infected *Culex sitiens*. The similarity of the erythrocytic stages of this parasite to those described for *P. juxtanucleare* has led us to term provisionally this parasite *P. juxtanucleare*. A survey of the chickens from the same region from where the infected mosquito was obtained showed that nine of them were infected with the same parasite. Three of 20 chickens in the IMR grounds were also infected naturally with the parasite.

CYCLE IN THE FOWL

Studies in progress in the Malayan *P. juxtanucleare* indicate a prepatent period of 14 days, a

high parasitaemia for 18-28 days and a long period of chronicity; the original infection still showing parasites some 2½ months following infection. Transfer of the parasites by blood inoculation has given variable results—all birds have become infected at times varying from 1-15 days following inoculation of infected blood. Birds inoculated with blood intravenously became infected more quickly than those inoculated subcutaneously.

CYCLE IN CULEX SITIENS

The parasite requires a minimum of 13.5 days to produce sporozoites and the oocysts do not begin to differentiate until about 11-12 days. The oocyst is unique in that it is pedunculated. The peduncle, first seen when the oocyst attains a diameter of about 30 micra (7-8 days), develops to a length of about 25 micra and about the same width. The oocyst then matures, attaining a diameter of 90-130 micra at differentiation; differentiation of sporozoites within the peduncle has not been observed. Following the release of the sporozoites and the disintegration of the oocyst, the peduncle persists for some time. Apparently the tissue in the peduncle differs from that of the oocyst; it has not yet been determined whether a septum divides the peduncle from the oocyst.

The erythrocytic stages, oocysts and sporozoites of *Plasmodium juxtanucleare* were demonstrated. In addition, sporozoites of *Plasmodium traguli* and *Plasmodium cynomolgi* in *Anopheles umbrosus* and *Anopheles maculatus* respectively were demonstrated in a comparison of different sporozoite types.

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Dr. J. W. Field, Mr. Yap Loy Fong and Dr. A. A. Sandosham

A RAPID METHOD OF STAINING THIN BLOOD FILMS FOR MALARIA DIAGNOSIS

MATERIAL AND METHODS

The requirements are (a) 0.2 per cent eosin (yellow, water soluble) in methanol (Analar), (b) Field's Stain 'A', and (c) phosphate buffer solution, pH 6.6.

(1) Put the thin film level on a staining rack, then pour 6 drops of methanolic eosin onto the film from a drop bottle.
(2) Immediately add 12 drops of Stain 'A'.
(3) Mix the stain by rocking the slide gently and stain for 1-5 minutes.
(4) Do *not* pour away the stain but wash by immersing the entire slide in clean running water or flush off with a jet of water from a wash bottle.
(5) Differentiate the stained film in phosphate solution, pH 6.6 for 5 seconds.
(6) Give the slide a final rinse in water, then allow it to drain and dry before examination.

RESULTS

The thin films stained by this method demonstrated compared favourably with those by the conventional Giemsa method which requires a longer staining time of half an hour or so. Schüffner's dots are easily demonstrable in the vivax-like *Plasmodium cynomolgi bastianellii* and Maurer's spots discernible in the falciparum-like *P. coatneyi* infected erythrocytes. The only minor drawbacks are that the films sometimes do not stain uniformly and occasionally they are bestrewn with stain debris despite the precaution taken in washing.

Mr. S. Sivanandam, Mr. Yap Loy Fong and Dr. A. A. Sandosham

A RAPID METHOD OF STAINING MICROFILARIAE IN THICK BLOOD FILMS

METHOD

When the thick blood film is dry after 30 minutes or longer (or even overnight), dehaemoglobinize it in water for two or three minutes. Stand the slide on end to drain and dry. Then fix the dehaemoglobinized film with methanol for a few seconds and dip the slide in water to wash away the methanol before staining.

(i) Immerse the film in Field's Stain 'A' for 1 second.
(ii) Wash in water for a few seconds.
(iii) Immerse in Field's Stain 'B' for 1 second.
(iv) Repeat wash in water.
(v) Counterstain in Stain 'A' for 1 second.
(vi) Final wash in water until excess stain disappears.
(vii) Place the slide on end to drain and dry.

RESULT

The thick films stained by this method appear to produce a better colour result and uniformity than by the Giemsa method. The microfilariae of *Brugia malayi* and *B. pahangi*, stain a brilliant purple with well preserved outlines and well-defined body and terminal nuclei; the sheaths show a brilliant stippled pink which stand out prominently against a background of deep purple leucocytes and pale pinkish lysed erythrocytes.

The microfilariae of *Wuchereria bancrofti* stain purple with well-defined discrete body nuclei and tapering tail. Usually it shows a characteristic unstained margin around the parasites and occasionally a faint pinkish sheath at the posterior extremity.

The routine thick-film rapid staining method for malaria diagnosis as described by Field (1941) usually fails to stain the microfilariae. This simple rapid method would provide the medical laboratory workers, especially in estates and during field surveys, with an easily available and rapid means of establishing diagnosis of filariasis other than depending on Giemsa or haematoxylin stains which require overnight drying and longer staining time.

The only noticeable drawbacks are the occasional contamination of the films with cellular or stain debris; films which are too thick will not dehaemoglobinize and stain well.

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Mr. G. L. Coombs and Dr. A. A. Sandosham

A MODIFICATION OF FIELD'S RAPID STAIN WHICH MAY BE USED IN STAINING THICK AND THIN BLOOD FILMS MADE SEPARATELY OR ON THE SAME SLIDE

MODIFICATIONS

The modification of Field's Stains recommended in this technique are that the stains are made in 0.85 per cent sodium chloride solution and the quantity of Azure I in Field's Stain 'A' is increased from 0.5 to 0.6 gm.

SOLUTION "A"

Methylene blue (Medicinal)	0.8 gm.
Azure 1	0.6 gm.
Disodium Hydrogen Phosphate Na_2HPO_4	5.0 gm.
Potassium Dihydrogen Phosphate KH_2PO_4	6.25 gm.
Sodium Chloride	4.25 gm.
Distilled water	500 ml.

SOLUTION "B"

Eosin	1.0 gm.
Disodium Hydrogen Phosphate Na_2HPO_4	5.0 gm.
Potassium Dihydrogen Phosphate KH_2PO_4	6.25 gm.
Sodium Chloride	4.25 gm.
Distilled water	500 ml.

TECHNIQUE OF STAINING THIN FILMS

Before staining the thin films have to be fixed by methanol and in using combined thin and thick films precaution has to be taken to prevent fixation of the thick film by contamination. During the standing and drying processes of the combined thin and thick films, the slide should be held with the thick film below.

METHOD OF STAINING

(1) Immerse the slide as soon as dry in solution "A" for 1 second.
(2) Rinse in running tap water until excess stain is removed.
(3) Immerse the film in Stain "B" for 2 to 3 seconds.

(4) Rinse as in (2).
(5) Immerse the film in Stain "A" for 10 to 15 seconds.
(6) Rinse as in (2) and place the slide to dry on a rack.

STAINING RESULTS

The vivax-like *Plasmodium cynomolgi bastianellii* and the falciparum-like *P. coatneyi* were used to test the stain and stained slides of these in thin and thick films were demonstrated.

In the thin blood films the erythrocytes stained a pinkish grey and did not appear overstained and the nuclei of the leucocytes appeared a deep purple. Trophozoites and schizonts appeared well stained with the chromatin a deep red and the cytoplasm a deep blue. The gametocytes appeared well stained with the males being a reddish pink and the females a pale blue. Blood films of *P. cynomolgi bastianellii* showed marked Schuffners stippling even with early trophozoites but in blood films of *P. coatneyi* Maurer's dots were only occasionally seen.

In the thick blood films the leucocytes stained a deep purplish blue, the chromatin a deep red and the cytoplasm a purplish blue on a pink background. Schüffner's stippling was also seen. No distortion to the parasites or the leucocytes was observed.

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By Prof. R. S. Desowitz and Dr. I. Polunin

THE INCIDENCE OF INTESTINAL PARASITES IN LAOS A PRELIMINARY REPORT

Very little is known regarding the incidence and nature of intestinal parasitism in Laos. As part of a general medical survey of the Laotian population one of us (I.P.) collected 90 stool samples from Pak Lay, a village about on the Mekong River. The stools were preserved in MIF fluid and sent by air to Singapore where they were examined. The present report gives the incidence of various parasites found by a single, direct-smear examination of the samples. The results indicate the high incidence of parasitism in the area. The stools are now being examined by concentration (MIF-C).

No. examined	90
No. positive for parasites	85 (94.5%)
Ascaris	72 (80.0%)
Hookworm	38 (42.2%)
Trichuris	6 (6.6%)
Strongyloides	7 (7.7%)
Taenia	6 (6.6%)
Clonorchis/Opistorchis	24 (26.6%)
Protozoan cysts	15 (16.6%)

By Dr. F. L. Dunn and Dr. J. M. Bolton

THE MIF DIRECT SMEAR (DS) METHOD IN THE STUDY OF INTESTINAL PARASITISM IN MALAYAN ABORIGINES

In August 1962 an investigation of intestinal parasitism in Malayan Aborigines was initiated by the Institute for Medical Research and the medical staff of the Department of Aborigines. The work has proceeded at three levels: 1) Continuing routine parasitologic diagnosis for all admissions (with signs, symptoms, or laboratory findings suggestive of intestinal parasitism) to the 100 bed Aborigines Hospital at Gombak near Kuala Lumpur. Treatment results are assessed by followup faecal examinations. 2) A survey of 200 consecutive admissions, for all causes, to the Gombak hospital—to provide preliminary information about the species of intestinal parasites occurring in Aborigines, and to establish crude prevalence baselines. 3) A series

of field surveys of selected Aboriginal populations in a variety of habitats. Five such surveys (covering 436 persons) have been completed to date. Each survey has been large enough, covering all age groups and both sexes, to serve as a statistically valid sample of the surveyed population. Three more surveys are planned. When these are completed, and all specimens have been studied, approximately 900 individuals (2% of the total population of c. 45,000) will have been examined at least once for intestinal parasites. The surveys will provide the following information (correlated with habitat and mode of life): 1) the kinds of parasites present, 2) prevalence rates for these parasites, and 3) data on intensity of infection for *Ascaris*, *Trichuris*, and hookworm (based on egg counts). The results of these surveys will provide guidelines for future mass treatment campaigns in the field. As a final phase of the investigation, pilot mass treatment projects will be carried out in several of the surveyed communities (with periodic resurvey to determine rates of reinfection and intensity of reinfection).

The basic procedure in all of these investigations is the *MIF-DS method* of examination of faecal specimens. The demonstration illustrates the steps involved in preparing MIF sets, and the procedures involved in handling and examining the specimens. The advantages and disadvantages of the procedure are outlined. It is emphasized that the MIF-DS method is invaluable not only for field survey use, but also for routine in small hospitals and other medical units in rural areas where laboratory facilities may be limited. The method is simple and, with proper precautions, at least as reliable as most of the well-known (and often more complex) procedures commonly in use. (The studies outlined above are being supported in part by US Public Health Service ICMRT Grant AI-04189-02; in part by the Office of the Surgeon General, Dept. of the Army.)

Appended is a list of parasites now known to occur in Malayan Aborigines. Those marked (*) have not previously been recorded in these people. *Balantidium coli*, and several other protozoa, have not previously been recorded from man in the Federation of Malaya (although they have been recognized in Singapore).

Helminths — hookworm, *Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, Taenia* sp., *Hymenolepis nana**, *Trichostrongylus* sp.* *Strongyloides stercoralis,* unknown dicrocoelid trematode—species.

1. *, unknown trematode—species 2. *, *Dipylidium caninum* has also been reported in Aborigines.

Protozoa — *Entamoeba histolytica,* large race; *Entamoeba histolytica,* small race = *E. hartmanni**, *Entamoeba coli**, *Endolimax nana**, *Iodamoeba bütschlii**, *Dientamoeba fragilis**, *Giardia lamblia**, *Chilomastix mesnili**, *Trichomonas hominis**, *Isospora* sp. *, *Balantidium coli* *.

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Mr. C. P. Ramachandran, Dr. F. L. Dunn, Professor A. A. Sandosham and Mr. S. Sivanandam

A *DIROFILARIA* FROM THE MUSANG

In the course of the filariasis investigations undertaken by the Institute for Medical Research in recent years, various wild and domestic animals have been examined in the East Pahang area of Malaya for infections with *Brugia* spp. The civet cat, *Paradoxurus hermaphroditus* (Carnivora), commonly known as the Musang, has been found infected in nature with subperiodic *Brugia malayi* and *Brugia pahangi* (Laing, Edeson, and Wharton, 1960). In addition Musangs have often been found infected with another filarial worm, usually recovered from the sub-cutaneous tissues. From very brief morphological studies (of microfilariae only) these worms have been regarded (Wharton, 1962) as *Dirofilaria repens*. No detailed studies of the

adult worm from Musangs have been undertaken in the past. In this demonstration some preliminary results of such a study are presented.

These worms conform in general characters to the Genus *Dirofilaria* (Railliet and Henry, 1911) which contains a number of species parastic in primates, carnivores, and rodents. Of these the best known are *Dirofilaria immitis* (Leidy, 1856), the heart worm of dogs, and *Dirofilaria repens* (Railliet and Henry, 1911) from cats and dogs. Webber (1955), and Chabaud and Rousselot (1956) have reviewed the *Dirofilaria* of primates. Price (1959) has described *Dirofilaria magnilarvatum*, from a Malayan *Macaca irus*. Rohde (1962) has studied the helminths of cats and dogs in Malaya and has reported *D. repens*-like nematodes from a domestic cat. Dr. Rohde of the Department of Zoology, University of Malaya has kindly permitted us to examine his specimens which conform in most respects to the classical description of *D. repens*. Both the males and females, however, are slightly shorter than in earlier descriptions.

None of the descriptions of *Dirofilaria* previously known correspond closely to the present forms recovered from the civet cat. Three complete males (and one fragment) and 14 complete females have been studied. The measurements, which are fairly consistent, indicate that these worms are much smaller in size than *D. immitis* and *D. repens*. It may be suggested that the size of the worm is only relative, and may vary from host to host. Size differences and proportions in structures such as the spicules, however, are less subject to variation. The left spicule in the genus *Dirofilaria* is divided into a proximal and a distal part. In *D. repens* the distal portion is about 1.5 times the length of the proximal, while in the Musang *Dirofilaria* the distal portion is less than, or approximately equal in length to the proximal portion. The whip-lash distal of the left spicule, characteristic of *D. repens*, is not present in the musang worms. These differences in form and structure, in conjunction with the smaller size of the adult worms, suggest to us that the Musang *Dirofilaria* is probably an undescribed species, distinct from but closely related to *D. repens*. The microfilariae resemble closely the published description of those of *D. repens* and *D. immitis*. Further work on this worm is continuing.

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Dr. K. Rohde (Introduced by Professor A. A. Sandosham)

TREMATODES OF BATS IN MALAYA (BAT AS HOST OF HUMAN PARASITE?)

42 bats, comprising 8 species, were examined for helminths. The following trematodes were encountered: *Cephalotrema* sp. (?) (*Prosthogonimidae*), *Postorchigenes duboisi* n.sp., *Lecithodendrium linstowi* Dollfus, 1931, *Prosthodendrium longiforme* Bhalerao, 1926, *P. parvouterus* Bhalerao, 1926, *P. swansoni* Macy 1936, *Odeningotrema bivesicularis* Rohde, 1962, and small unidentified Lecithodendriidae. All species except the first belong to the family Lecithodendriidae. *Postorchigenes duboisi* n.sp. is the first member of this genus from mammals. Of special interest is the finding of *Odeningotrema bivesicularis* in a bat. This indicates that the genus *Odeningotrema* Rohde, 1962, subfam. Odeningotrematinae Rohde, 1962, has a very low degree of host-specificity. It has been found in Primates (Tupaiidae, Lorisidae), Chiroptera (Molossidae), and Insectivora (Erinaceidae). (Rohde, in press).

In the meantime, a large number of trematodes from 5 specimens of a 9th species of bats, *Taphozous melanopogon*, were examined. Four species of Lecithodendriidae, belonging to the genus *Prosthodendrium* (2 of them to the subgenus *Paralecithodendrium*) were found. Most

common is a form closely resembling *P.* (*Paralecithodendrium*) *molenkampi* (Lie-Kian-Joe, 1951). It is very probably identical with this species, which was described as a species of the genus *Paralecithodendrium* from man in Indonesia and later (comp. Dubois, 1962) put into the genus *Prosthodendrium*, subgenus *Paralecithodendrium* (see exhibit and figure 11). Thus, *Taphozous* must be considered a reservoir host of this (accidental?) human parasite.

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Dr. Hong-Fang Lee

TWO NEW TREMATODES OF FERAL RATS IN MALAYA AND NORTH BORNEO

Five species of trematodes were recovered from feral rats trapped in Malaya and North Borneo. *Skrjabinus muris* Stscherbakova, 1942 and *Athesmia foxi* Goldberger and Crane, 1911 (both Dicrocoeliidae), were found for the first time in Malaya and the rats (*R. sabanus, R. argentiventer* and *R. rajah surifer*) represented new hosts; *Echinostoma malayanum*, a common intestinal fluke of Malayan rats, was found in *R. argentiventer*. Two new species of a new genus, tentatively assigned to the family Troglotrematidae, were recovered from *R. sabanus* and *R. infraluteus*.

The two new trematodes do not belong to any of the described genera. The position of the genital pore is similar to that in the genus *Prostho-gonimus*, but the structure and position of the other organs exclude them from this genus. They resemble *Paragonimus* in spination, position of ovary, testes, uterus and distribution of vitellaria, but they differ in that they have smaller eggs and Y-shaped excretory vesicle. The generic name *Beavertrema* is proposed for these species in honor of Professor Paul C. Beaver of Tulane University.

Beavertrema beaveri was recovered from both liver and intestines of *R. sabanus* trapped in Malaya while *B. microacetabulum* was found only in the liver of *R. infraluteus* from North Borneo. *B. microacetabulum* is distinguished from *B. beaveri* by a smaller acetabulum which is closer to the oral sucker.

Dr. P. F. Basch (Introduced by Prof. A. A. Sandosham)

AN AVIAN SCHISTOSOME FROM NEGRI SEMBILAN PADI FIELDS

Collections of freshwater snails, Lymnaea sp., from padi fields in the vicinity of Kuala Pilah, Negri Sembilan, have revealed a great many trematodes present in the fields. There are several species of echinostomes, at least one small stylet cercaria, at least two strigeids, and an eyed schistosome cercaria of the "cercaria ocellata" group. This latter cercaria is capable of causing a dermatitis in man, as was pointed out by Dr. Sandosham in 1953 (Malaysian Parasites XII). Large numbers of snails have been collected and checked, and about 12 naturally infected snails are regularly shedding these cercariae in the laboratory. Experimental exposures have been made to ducks, doves, sparrows, munias, finches, and mice, but thus far only ducklings have proved to be suitable hosts, as shown by miracidia hatched from their faeces. Of five mature ducks purchased in Kuala Pilah, one had a light infection, and one a very slight case, as demonstrated by hatching of miracidia. One experimental duckling has been sacrificed, and in spite of an intensive search no adult worms were located; more animals are now being given very heavy exposures in hopes on obtaining the adult worms and eggs. The adult seems to be a species of *Trichobilharzia*. One observation of interest in the laboratory has been the finding in small free-living rhabdocoel flatworms of numbers of cercariae, alive and moving. Whether the worms eat the cercariae, or whether the latter penetrate actively is still not known. Attempts have been

made to establish the life cycle in the laboratory by exposing clean lab-raised snails to miracidia from naturally and experimentally infected ducks.

Any specimens or information about avian or mammalian schistosomes in Malaysia would be gratefully received by the writer at the address above.

Mr. Lim Boo Liat, Mr. Ow-Yang Chee Kong and Prof. Lie Kian Joe
(Introduced by Prof. A. A. Sandosham)

FURTHER RESULTS OF STUDIES ON *ANGIOSTRONGYLUS CANTONENSIS* CHEN, 1935 IN MALAYA

Angiostrongylus cantonensis has been found in rats in various habitats around Kuala Lumpur: scrub, lallang, rice field and oil palm estates. Land snails and slugs collected from these areas were dissected and the results were shown in tables 1, 2, 3 and 4. Third stage larvae were found in *Microparmarion malayanum, Parmarion* sp. and *Pseudoplecto bijuga.*

It is found that the rats do swallow the slugs and land snails in the laboratory as well as in the field. Moreover, rat faeces seem to form part of the food taken by land snails and slugs. The relationship between rats and the intermediate hosts is therefore direct and mutual.

In addition, third stage larvae were found in fresh water snails collected from padi fields. Results of dissections of these snails were shown in table 5. These fresh water snails can be infected under experimental conditions in the laboratory. They may be infected with first stage larvae which will develop into third stage larvae in these snails. They may also be infected with third stage larvae which remain as such in the snail without further development. Table 6 showed the results of experiments with first stage larvae and table 7 with third stage larvae.

Fresh water snails do eat dead slugs and land snails. Infection of fresh water snails in nature may occur from eating dead slugs or land snails or perhaps from infection with first stage larvae. *Pila scutata* and *Bellamya* snails are eaten by man. In certain countries they are eaten raw. They are therefore a possible source for human infection with *Angiostrongylus cantonensis.* Attempts to infect fresh-water shrimps with larvae of *Angiostrongylus* have failed.

Prof. Lie Kian Joe (Introduced by Prof. A. A. Sandosham)

THE LIFE HISTORY OF A *HYPODERAEUM* sp. (TREMATODA: ECHINOSTOMATIDAE)

The complete life cycle of a *Hypoderaeum species* a parasite of ducks and geese, has been worked out. The freshwater snail *Lymnaea rubiginosa* serves as the first intermediate host. The sporocyst is found in the heart muscle. There are at least 3 redial generations. The second intermediate hosts are different freshwater snails (*L. rubiginosa, Gyraulus convexiusculus, Indoplanorbis exustus*) and tadpoles (*Rhacophorus leucomystax*). Drawings were shown of the sporocyst, mother redia, redia containing rediae and cercariae, cercaria, and metacercariariae. A stained adult worm was also shown. The parasite is described as a new species in Tropical and Geographical Medicine (*in press*).

Mr. Cheong Wèng Hooi and Mr. C. P. Ramachandran
(Introduced by Prof. A. A. Sandosham)

(MERMETHID?) FROM TWO MALAYAN MOSQUITOES

During the course of our entomological investigations on Malaria and Filariasis, various filarial and nematode worms of unknown generic or specific identity have been recovered from mosquitoes. As far as we know, no previous reports have been made on these findings. The present demonstrations show two of such unknown nematodes recovered from:—

(a) *Anopheles letifer*: Wild caught at Bukit Kelubi in Selangor on 22/11/61. The unknown nematode was found in the abdominal haemocoele.

See slide 1.
Length: 17.35 m.m.
Breadth: 0.12 m.m.

(b) *Aedes albopictus*: Wild caught at Ulu Gombak in Selangor on 8.6.63. The unknown nematode was recovered from the abdominal haemocoele.

See slide 2.
Length: 11.1 m.m.
Breadth: 0.145 m.m.

A number of workers have reported nematode parasitism of adult mosquitoes as well as in the larval forms. *Agamomermis sp.* have been collected from the abdominal cavity of larvae, pupae and adults of *Culex nemorales* Stiles (1903). Smith (1904) have reported the occurrence of *Agamomermis culicis* from *Aedes sollicitans* in New Jersey. Many species of mermithid nematodes have been reported from mosquitoes but little is known of their biology. Lyengar (1930) has also noted the occurrence of certain large species of *Mermis* from adult mosquitoes. A mermithid nematode was described in *Aedes communis* in Canada, Jenkins and West. (1954).

These appear to be the first records of rather similar infections from Malaya.

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Prof. Lie Kian Joe (Introduced by Prof. A. A. Sandosham)

THE LIFE HISTORY OF *ECHINOSTOMA LINDOENSE* SANDGROUND AND BONNE, 1940 (TREMATODA; ECHINOSTOMATIDAE)

Echinostoma lindoense is a human parasite found among the inhabitants around Lake Lindoe in Central Celebes, Indonesia (Sandground and Bonne, *Am. J. Trop. Med.*, 1940, *20*, 511). The life cycle of the parasite in that region was partly worked out. The first intermediate host is *Gyraulus sarasinorum* and the second intermediate hosts are various freshwater snails and a mussel *Corbicula lindoensis* Boll. Man gets infected by eating the infected mussels.

E. lindoense is now found in Malaya, the first record of *E. lindoense*, outside the Lindoe area. The complete life cycle is worked out. The freshwater snail *Gyraulus convexiusculus* serves as first intermediate host. The sporocyst develops in the heart muscle. There are 2 redial generations. The second intermediate hosts are freshwater snails (*Lymnaea rubiginosa, Gyraulus convexiusculus, Indoplanorbis exustus*) and tadpoles (*Rhacophorus leucomystax*). The parasite is maintained in nature in Malaya by domestic ducks and probably also geese. White mice are suitable experimental hosts. The parasite is not reported in man yet in this country. Drawings of a sporocyst, mother redia, daughter redia, cercaria, and metacercaria were shown. A stained adult worm was also demonstrated.

Mr. Ow Yang Chee Kong (Introduced by Prof. A. A. Sandosham)

A SPECIES OF *HAPLORCHIS* FROM THE WATER MONITER

Since the establishment of *Procerovum* by Orji and Nishio (1924) and *Haplorchoides* by Chen (1949), many species of heterophyid trematodes that had been referred to the genus *Haplorchis* Looss, were transferred to these two genera. On the basis of this reclassification, only 6 species that Morozov (1952) listed as distinct, still remain in the original genus.

A species of *Haplorchis* Looss, s.s. which does not appear to fit into any of the existing species has now been found in *Varanus salvator* (Laurenti) from Malacca. Incidentally, this is also the first record from a reptilian host, those reported previously being from warm blooded animals only; including at least 4 species from man—namely, *H. Pumilio* (Looss), *H. taichui* (Nishigori), *H. vanissimus* Africa, and *H. yokogawai* (Katsuta) (vide Yamaguti, 1961).

A stained specimen of this worm was exhibited.

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Dr. K. Rohde (Introduced by Professor A. A. Sandosham)

STUDIES ON THE GENITAL SYSTEM OF MONOGENETIC TREMATODES

Recently the author described the first Malayan monogenetic trematode, belonging to the genus *Polystomoides* Ward, 1917, from the urinary bladder of the tortoise *Cyclemys amboinensis* (Daud.) (Rohde, 1963; see exhibit 1 and figure 1). In the meantime, 3 further species of the same genus have been found. Descriptions of these species and a revision of the whole genus are being prepared.

The presence of 4 species of the same genus in Malaya, occurring in sufficient numbers, appears to provide a good opportunity for comparative histological and cytological studies of Monogenea. Histology and cytology of this group are very poorly known (Bychowsky 1957).

In the following, some preliminary results of comparative histological studies on the ootype, and the accessory male glands of *Polystomoides* spp. are communicated.

(A) *Ootype.* In the largest species (*P. malayi*), there are mucous gland cells, which open into the ductus communis just in front of its entrance into the ootype (figures 2, 3), and large serous glands with huge nuclei, which open into the main part of the ootype. In addition, numerous small nuclei of the ordinary parenchymatous type are distributed mainly in the connective tissue, situated between the serous glands, and around the anterior and posterior entrances into the ootype. Similar nuclei are in the epithelium of the ootype. As a working hypothesis it is assumed that these nuclei migrate into the ootype, forming the (temporary?, see below) epithelium, which is eventually shed into the lumen of the ootype (figures 4, 5, 6). — According to Kohlmann (1961) the same types of cells can be found in the closely related form *Polystomminteger-rimum* Fröhlich: mucous glands at the entrance of the ductus communis into the ootype, and serous glands opening into the ootype, which is surrounded by an epithelium.

In the smaller species of *Polystomoides* mucous gland cells around the entrance of the ductus communis into the ootype and numerous small, undifferentiated nuclei around the ootype are also present. But though serous secretion can always be found in the lumen of the ootype, serous gland cells can usually not or only with difficulty be distinguished. Even if they are distinguishable, they are much smaller and much

more similar to the ordinary parenchymatous type of cells than the serous glands in *P. malayi.*

A continuous epithelium around the ootype of at least the 2 smallest species is absent, though there are always a few nuclei inside the lumen, which have probably immigrated from the surrounding tissue (see above, figure 8).

The structure of the ootype in the 3 small species of Polystomoides appears to correspond in many points to the structure of the ootype in immature *P. malayi* (figure 7).

(B) *Accessory male glands.* The same variability among the 4 species of *Polystomoides* can be found in the accessory male glands. *P. malayi,* the largest species, has two types of accessory glands, i.e. mucous and serous glands. The second largest species has only 1 type (or perhaps 2 types which are very similar to each other; not yet verified!) resembling the serous glands of *P. malayi*! The 2 smallest species have no morphologically distinct accessory male glands at all, though in these cases there is always a cluster of small nuclei, similar to the parenchymatous ones, near the genital bulb. *Polystomum integerrimum,* according to Kohlmann, 1961, has only 1 type of accessory male glands.

Hence, the presence of certain types of ootype glands and accessory male glands in *Polystoma* and *Polystomoides* is not a generic character, but varies greatly, depending on the size of the parasite.

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Mrs. V. Thomas (Introduced by Prof. A. A. Sandosham)

THE VARIATION IN DDT-RESISTANCE IN LARVAE OF DIRECTLY AND INDIRECTLY SELECTED COLONIES OF *CULEX PIPIENS FATIGANS* WIED

Two methods of selection were adopted. One was by direct selection when larvae were hatched out in bulk from many egg rafts obtained from Klang and submitted to a discrimination concentration of 5 p.p.m. of DDT. The adults that emerged from the survivors were maintained as a resistant colony and their larvae in successive generations were subjected to the same concentration over a number of generations.

The other method of selection was an indirect one. Larvae hatched out from individual egg rafts collected from Klang were reared separately and about 50 larvae from each raft were subjected to 5 p.p.m. of DDT to determine the mortality rate. The remaining unexposed larvae of individual batches that showed the lowest mortality rate to this concentration were maintained as separate colonies for several generations. Three colonies were established and changes in mortality rate in successive generations were observed on about 50 larvae of 10 individual rafts from these colonies. In this process of indirect selection the larvae which gave rise to the subsequent generations had not been subjected to selection by exposure to DDT.

In the first colony where the larvae were isolated and maintained under DDT-selection pressure in successive generations, resistance to DDT rose to a high level. In the colonies of the second group which had been selected solely because of high resistance of their sibs to DDT, the resistance was maintained only through the first 2 to 4 generations and subsequently lost completely by the 5th generation (see graph). The LC_{50} value in the directly selected colony was 14.5 p.p.m. for the sixth generation whereas it had fallen in the indirectly selected colonies to 0.33 and 0.28 p.p.m. by the fifth generation.

It was noted that the building up of resistance even under selection pressure was fluctuating and irregular and that there was rapid reversion to susceptibility in untreated larvae. These experimental findings suggest that DDT-resistance in larvae of *C. p. fatigans* is due to cytoplasmic modification and were confirmed in reciprocal and back-cross experiments.