LABORATORY DEMONSTRATIONS

LABORATORY DEMONSTRATION MEETINGS

Held under the auspices of the Malaysian Society of Parasitology and Tropical Medicine at the Institute for Medical Research, Kuala Lumpur on January 11th, 1969 and July 5th, 1969.

Edited by C.P. RAMACHANDRAN

The demonstrations were set up by the staff of:—

INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR
- Division of Filariasis Research
- Division of Malaria Research
- Division of Vertebrate Zoology and Bio-Medical Museum
- Division of Medical Entomology
- G.W. Hooper Foundation

UNIVERSITY OF MALAYA, KUALA LUMPUR
- Department of Parasitology
- School of Biological Sciences

GOMBAK ABORIGINE HOSPITAL, KUALA LUMPUR

UNIVERSITY OF SINGAPORE
- Department of Parasitology

CANNIBALISM IN *ENTAMOEBA INVADENS*

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Entamoeba trophozoites, in addition to ingesting red cells and starch, are also known to ingest their own cysts. In old cultures where starch has been depleted by amoebae it is not uncommon to find *E. invadens* trophozoites with ingested cysts. This observation has been made previously by Geiman and Ratcliffe (1936) and by McConnachie (1955). However, up to now there has been no report of trophozoites ingesting trophozoites of its own species. A culture of *E. invadens* was recently observed by the author in which a number of large trophozoites were seen which had in their cytoplasm actively moving, but smaller trophozoites (Fig. 1). The trophozoite which had ingested other trophozoites usually had no starch in the cytoplasm. It would seem that cannibalism occurs when food in particulate form e.g. starch, is no longer available. Observations made over an hour showed that the ingested trophozoites remained active in the cytoplasm of the trophozoites which had ingested them. It is not known whether the ingested trophozoites are finally broken down and digested or expelled in an undigested form.

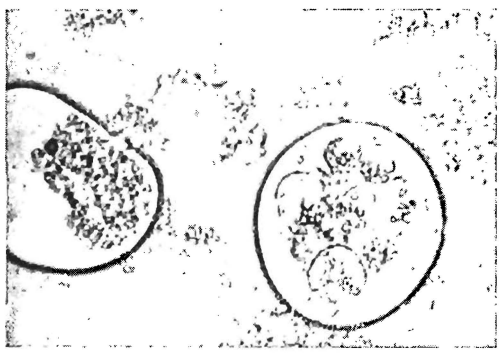

Fig. 1— Showing a large trophozoite with a number of actively moving smaller trophozoites in its cytoplasm.

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BALANTIDIUM COLI (MALMSTEN, 1857) IN PIGS SLAUGHTERED AT THE KUALA LUMPUR ABBATOIR

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Balantidium coli is the only pathogenic ciliate of man living in the colon and caecum. It is also a very common parasite in the intestine of pigs in which animals it is relatively harmless. *B. coli* can invade and ulcerate the intestinal wall of pigs if these animals have a superadded *Salmonella* infection or are run down for any reason. Human infection, which is mainly by swallowing cysts in contaminated food or water is largely associated with pigs.

B. coli is also a parasite in the intestine of the chimpanzee and orang-utan. In Malaysia it has been found in gibbons (Ellefson, unpublished and quoted by Sandosham and Keling, 1967). Examination of stools for *B. coli* infection of 247 pigs reared predominantly by the Chinese and brought to the Kuala Lumpur abbatoir for slaughter showed 114 animals (46 per cent) were positive.

There is only one record of human infection with *B. coli* in Malaysia, which is that of Dunn (1966) who found that 3 out of 4 infections were among the Semelai, the only pig-rearing Aborigines in Malaya. Physicians in Malaysia should look out for the infections especially in those with dysentery and frank blood and mucus in the stools. In Singapore the infections recorded were mostly amongst the Chinese.

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A MIXED INFECTION OF *PLASMODIUM MALARIAE, P. VIVAX* AND *P. FALCIPARUM* IN A MALAY CHILD

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During a parasitological survey of Pulau Perhentian Kechil, off Trengganu, in West Malaysia, (Yap *et al.* 1969) in April, May 1967, a $5\frac{1}{2}$ year old Malay boy was found with a mixed infection of 3 species of human plasmodia where the malaria was hyperendemic on the island.

The Giemsa-stained thick-blood film, under demonstration, showed asexual and sexual forms of *P. malariae,* late trophozoites of *P. vivax* and gametocytes of *P. falciparum.*

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TOXOPLASMA INFECTION IN PIGS IN KUALA LUMPUR ABATTOIR

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Toxoplasma, a protozoan which is widely distributed throughout the world, infects not only man but many species of animals. Many individuals of each species may be infected. It generally behaves as a well adapted, harmless parasite depending on the species of animal but sometimes it does produce disease. The virulence depends on the strain of parasite. Serological surveys indicate that the incidence in animals varies from country to country and from district to district. Beattie (1963) reported that in 1961 he failed to isolate *Toxoplasma* with certainty from any of the 120 unselected human brains of which only a small portion of each was examined.

32 pig brains were collected from the Kuala Lumpur abattoir in 1968. A small portion of the brain was removed for histological study. Examination of these did not reveal any *Toxoplasma.* A further portion (5G) of the brain was emulsified in sterile saline, 3 parts by volume to which had been added sodium benzyl penicillin, 20,000i.u. and streptomycin sulphate 16,000 i.u. per ml. of saline. The emulsion was allowed to stand for 1 hour. No concentration was carried out and a random sample of 0.5 ml. of the emulsion was injected intraperitoneally into each of 2 mice. The mice were examined at intervals from 1 week to 3 months after inoculation. No *Toxoplasma* organisms were isolated from them. Their brains were collected and inoculated into new mice. Brain, spleen and liver smears from these mice were negative for *Toxoplasma.*

Singh *et al.* (1967) using the indirect haemagglutination (IHA) technique found 6 (12.5%) out of 48 pig sera from Malaya positive for *Toxoplasma* antibodies. A similar test carried out on pig sera from Singapore showed 56 (27.7%) out of 202 positive. Our failure to isolate the parasite should not be set against the possibility that latent *Toxoplasma* infection is relatively common in pigs as revealed by serological tests.

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TRYPANOSOMA EVANSI AND *ECHINOCOCCUS GRANULOSUS* IN A CAMEL *(CAMELUS DROMEDARIUS)* AT ZOO NEGARA

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Clinical History

A nine year old dromedary was one of four camels which arrived at Zoo Negara, Kuala Lumpur, from West Pakistan on 28/8/63. Shortly after arrival the animal was found to be infested with nematodes, and *Trypanosoma evansi* infection was detected by examination of peripheral blood films. The camel regained good health following deworming, and trypanosomes were not seen in blood films after one dose of Antrycide was given.

On 9/5/68 the camel developed a watery type of diarrhoea. Deworming and symptomatic treatment of the diarrhoea did not result in recovery, and the camel went off feed, continued to lose weight, developed a keratoconjunctivitis and melaena. Subsequent blood examinations revealed a moderate anaemia and the presence of *Trypanosoma evansi* in large numbers. One gram of Naganol (Bayer 205) in 15 cc Antimosan solution was given intravenously.

The camel was found dead on the morning of 14/6/68.

Necropsy Findings

The major post-mortem lesions were as follows: acute fibrinous perirenal peritonitis (left kidney only) with adhesions of a small portion of the greater omentum, petecheal hemorrhages in the epicardium

of the right and left ventricles and in the peripheral pleura of the diaphragm, sanguinous thoracic fluid (approximately $\frac{1}{2}$ L.), severe pulmonary congestion and edema and massive atalectasis with necrosis, large numbers of "hydatid" cysts throughout the lungs, and the liver contained a few large cysts and was cirrhotic.

The spherical cysts were unilocular, with high internal pressure and numerous folds of the germinal epithelium, and filled with "hydatid sand" floating in clear, pale yellow, vesicular fluid. Five of the pulmonary cysts, ranging from approximately 2 cm to 8 cm in diameter, were dissected, and none of them were "sterile".

Discussion

There is no doubt that these two parasitic diseases were imported to Malaysia from abroad by a host which appeared healthy at the time of shipment.

The discovery of the "Surra" organism soon after arrival at the Zoo indicated that the camel was a "carrier". It is likely that the shipping stresses and the heavy helminth load unmasked the trypanosome infection. The clinical trypanosomiasis five years later which led to the animal's death could have been autogenous from the "latent" infection within. The failure of the Antrycide injection to eliminate the carrier state could most likely have been indicated by inoculation of a susceptible laboratory host (e.g., rats) with blood samples taken over a period of several days.

That the camel represented a possible source for transmission of the trypanosomes to the other camels in the Zoo and to other natural hosts (such as oxen, buffalo, and dogs) which are numerous in the vicinity outside the Zoo is a concrete possibility. Blood-sucking flies (*Tabanus* spp. and *Chrysops* spp.), which are potential vectors, were caught in fairly large numbers in and around the Zoo compound. *Trypanosoma evansi* infections have been detected among dogs in the Ulu Klang region not too far from Zoo Negara, which indicated that the disease was endemic in the district. The camel did not represent a possible economic threat to domestic livestock which frequently are herded past the Zoo since "Surra" in cattle is mild and recovery is almost invariably the outcome.

Natural echinococcosis is not known to be endemic in Malaysia as far as we know but infected animals may gain entrance into the country, as illustrated by the camel at Zoo Negara. *Echinococcus granulosus* is known to infect camels in West Pakistan. Echinococcosis is usually discovered only upon necropsy, however the hosts may harbour the parasite for many years. Although the space-occupying lesions in the Zoo camel certainly was responsible for extensive pulmonary and hepatic tissue damage, clinical signs of respiratory distress, chronic emaciation, or specific hepatic disease were not observed during the course of the infection. No doubt the "hydatid" disease was "active" at the time of death and was a stressor factor, but the clinical syndrome seen in this case was typical of acute trypanosomiasis as seen in camels.

MICROFILARAEMIA LEVELS IN CATS AFTER SINGLE AND MULTIPLE INFECTIONS WITH *BRUGIA PAHANGI*

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The aim of this study was to compare microfilaraemia levels in cats after single and multiple infections with *Brugia pahangi*. The cats were divided into three groups-A, B and C. Group A received a single injection of $50L_3$ larvae (see Fig. 1). Group B and C received 3 and 6 injections each of $50L_3$ larvae, given at intervals, as shown in Figs. 2 and 3 respectively. The L_3 larvae were obtained by dissecting *Armigeres subalbatus* that have been previously fed on cats infected with *B. pahangi*. They were injected subcutaneously into the hind legs of cats using a wide-bore needle. Subsequently the cats were examined at monthly intervals by drawing out 20 c.mm. of blood and staining with Giemsa.

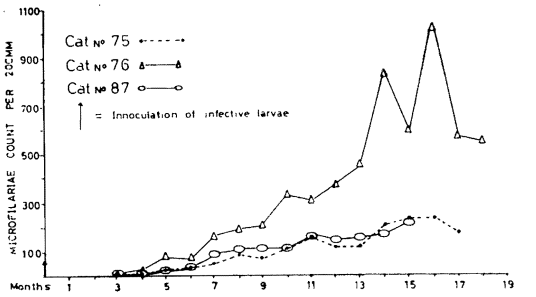

Fig. 1

Fig. 1 shows microfilaraemia levels in three cats (75, 76 and 87). In one cat (No. 76) microfilaraemia reached a peak of approximately 1000 per 20 c.mm. in about 16 months after the date of infection. In the other 2 cats microfilaraemia remained more or less constant at about 150 per 20 c.mm.

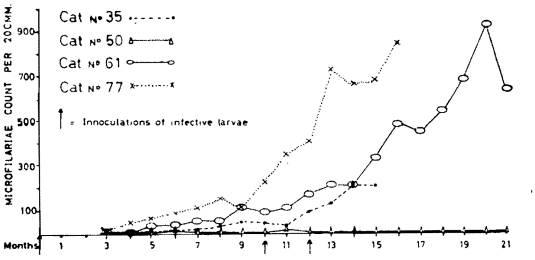

Fig. 2

Fig. 2 shows microfilaraemia levels in four cats (35, 50, 61 and 77) belonging to Group B. Cat (No.61) showed a peak microfilaraemia nearing 1000 per 20 c.mm., which subsequently declined to approximately 650 per 20 c.mm. Cat (No. 77) showed a peak of approximately 850 mf. per 20 c.mm. and cat (No. 35) showed a peak of approximately 200 mf. per 20 c.mm. Cat (No. 50) showed 2 very insignificant transitory microfilaraemia levels after the first and second injection. It then remained completely negative throughout the period of study.

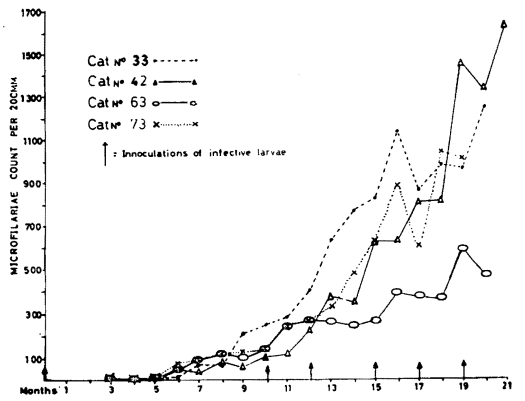

Fig. 3

In Group C (see Fig. 3) cat (No. 42) showed a peak level of approximately 1700 mf. per 20 c.mm. which to our knowledge is a record number for *B. pahangi*. Cat (No. 33) showed a peak of approximately 1200 mf. per 20 c.mm.; cat (No. 73) a peak of approximately 1000 mf. per 20 c.mm. and cat (No. 63) a peak of 600 mf. per 20 c.mm.

This study shows that there is a great deal of variation in the levels of microfilaraemia observed in different animals over a period of time. In cat (No. 76) belonging to Group A, after a single infection microfilaraemia reached a very high level of approximately 1000/20 c.mm. while in cat (No. 50) even after 3 infections, there was no microfilaraemia, with the exception of 2 small transitory peaks. The reason for this extraordinary variation is not clear. Although the immunological status of these cats was not known, all the animals had come from Singapore, where natural infection with *B. pahangi* has not been recorded. If the low levels of microfilaraemia in some of these cats is due to immune response, it is more likely to factors connected with natural, rather than acquired immunity. The rising levels of microfilaraemia in cat 42, 73 and 33, even after 6 infections indicate that acquired immunity does not play an important role in the control of microfilaraemia, at least in some of the animals.

FILARIAL WORM IN CYST OF BREAST

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The slide under demonstration showed a section through a cyst in the skin of the breast of an adult Chinese woman which was surgically removed in Penang.

Observed in the section was a longitudinal section of a filarial worm. *Wuchereria bancrofti* is occasionally found in cutaneous cysts in Malaya and this may be another such case. No information is available as to the period of residence of the patient in the country and whether she had any microfilariaemia in her blood.

ASCARIS EGGS IN PANCREAS

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The demonstration showed a section of pancreas removed surgically from a 30-year old woman at Miri, Sarawak, with several *Ascaris* eggs, some decortiated, embedded in eosinophilic granuloma in pancreatic tissue.

No history of the case was available but Dr. T.R. Arulampalam reports that the specimen which was removed surgically consisted of a loop of duodenum, pancreas and spleen. The duodenum was grossly thickened and adherent to the head of the pancreas which was firm and hard suggestive of chronic pancreatitis.

The slide demonstrated is of interest because the presence of *Ascaris* eggs embedded in the pancreatic tissues is extremely rare. The massive infiltration of pancreatic tissue by eosinophiles is also considered to be unique.

NATURAL INFECTION OF THE LAND MOLLUSC *ACHATINA FULICA* WITH *ANGIOSTRONGYLUS CANTONENSIS* IN MALAYA

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Achatina fulica is of East African origin and during the 19th century these snails have moved to South East Asia and were found in Malaya in 1911 (Mead 1961). The role of *Achatina* snails in the spread of *A. cantonensis* the causative agent of "eosinophilic meningitis" in parts of Southeast Asia and the Pacific area is unknown. Alicata (1965) has found this snail very susceptible to infection with larvae of *A. cantonensis* and found 100% infection rates in the Hawaiian Islands, Ponape and Saipan with approximate number of larvae varying from 208-1, 054 in the snails from the Hawaiian Islands. Lim and Heyneman (1965) examined 860 *A. fulica* snails from urban and rural areas of central Malaya and found only 12 (1.4 per cent) infected.

82 *A. fulica* snails were examined and 22 snails (26.8%) harboured third stage *A. cantonensis* larvae, mainly around the region of the hepato-pancreas. Of these snails, 49 were collected from areas in and around Kuala Lumpur and 13 (26.5%) were found infected. 3 (16.7%) out of 18 snails were infected from a rubber estate and 6 (40%) out of 15 were infected in an isolated rural village, Bukit Tinggi, Pahang State. The number of larvae per snail varied from 7 to 600. The larvae collected from some of the snails were fed to 3 albino rats and in all these animals adults of *A. cantonensis* were recovered from the pulmonary arteries.

Lim and Heyneman (1965) found rat's nests with broken snail shells of *A. fulica* and *Bradybaena*

similaris. These authors in an examination of 1,100 of the latter snails found no case of *A. cantonensis* infection among them. *A. fulica* is widespread in both urban and rural areas of Malaya and in urban areas is commonly seen after the rains in gardens. Together with other species of land snails and slugs, *A. fulica* form a significant source of infection of rats with *A. cantonensis* in Malaya.

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PRELIMINARY SURVEY FOR *ANGIOSTRONGYLUS CANTONENSIS* IN EDIBLE FRESH-WATER SNAILS IN WEST MALAYSIA

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In Thailand fresh-water snails particularly *Pila* spp. were found to be one of the important intermediate hosts of *Angiostrongylus cantonensis* which reflected the epidemiology of human eosinophilic meningoencephalitis (Punyagupta, 1965). The disease has not yet been reported in West Malaysia although the parasite is widely distributed throughout the country. Fresh-water snails, *Pila scutata*, *Bellamyia ingallsiana* and *Indoplanorbis exutus* collected in a ricefield were found naturally infected, but the infection rates and worm loads in these snails were insignificant as compared to the land molluscs, the principal intermediate hosts of the parasite (Lim *et al.* 1965) The present study deals with four species of fresh-water snails, *Pila scutata*, *Bellamyia ingallsiana*, *Brotia costula* and *Tiara* sp. which have been known to be eaten by humans occasionally as food as well as for medicinal purposes by certain communities in West Malaysia. Attempts were made to determine whether these snails serve as intermediate and carrier hosts of the parasite. This knowledge may be of epidemiological importance. Heyneman and Lim (1967) had earlier demonstrated that ingesting raw lettuce could be another mode of human infection for *Angiostrongylus cantonensis*.

A total of 982 molluscs consisting of 393 *Pila scutata*, 178 *Bellamyia ingallsiana*, 251 *Brotia costula* and 160 *Tiara* sp. collected in mining pools and fish ponds within the municipal areas around Kuala Lumpur were examined. None of these snails were found to have natural infections of the parasites.

Experimental infections in the four said species of fresh-water snails were carried out. The snails used for the experiments were obtained from the same areas as those previously examined and found free of infection.

Table 1 shows the results of direct feeding of naturally infected land molluscs *Microparmarion malayanus and Machrochlamys resplendens* which were cut up and fed to the experimental snails: *Pila scutata*, *Bellamyia ingallsiana*, *Brotia costula* and *Tiara* sp. Fifty snails of each seperate aquaria were used. Daily, the snails in each aquarium were fed on three land molluscs. The snails were dissected 5-10 days after the last feeding. Results of the experiments show that 60% (30/50) *Pila scutata* and 10% (5/50) *Bellamyia ingallsiana* were found with third-stage larvae in the viscera and only a few larvae were found in the masculature. The total number of third-stage larvae recovered from these experimental *Pila* snails was 1176 with an average of 59 and a range from 2-463 larvae per snail. These larvae were innoculated to four experimental white rats. Five sub-adult worms were recovered in the brain of one rat that died 15 days after the infection. The second rat killed 30 days after the infection was found with two adult worms in the brain, 25 in the heart and six in the lungs. The remaining two experimental white rats were still living 35 days after infection.

Only 23 third-stage larvae were recovered from the five *Bellamyia* snails dissected. This species averaged four larvae per snail, with a range from 1-12. The larvae were fed to two experimental white rats. The first rat killed 30 days after the infection was found with one adult worm in the heart and two in the lungs. This showed that the third-stage larvae from naturally infected molluscs ingested by these experimental snails were still viable and capable of infecting the rat hosts.

Brotia costula and *Tiara* sp. did not feed on the infected land molluscs offered and hence none was found positive on dissections for *angiostrongylus cantonensis* larvae.

Table 2 shows experimental infections in the four species of fresh-water snails infected with faeces obtained from experimental white rats. The number of snails used for each species was 50 and they were kept in separate aquaria. The snails in each aquarium were given five pieces of rat faeces infected with first-stage larvae everyday for a period of 10 days. The snails were dissected 24-30 days after the last feeding. Results of the experiments show that 4% (2/50) *Pila scutata* became infected. Only 10 infective larvae were recovered from the muscle tissues from two snails (3 and 7) respectively. The larvae were fed to one experimental white rat and was killed 30 days after infection. Two adult worms were found in the heart. The rest of the three species of fresh-water snails failed to become infected.

The preliminary results of these experiments in the four species of edible fresh-water snails indicate that at least two species *Pila scutata* and *Bellamyia ingallsiana* can serve as paratenic hosts for *Angiostrongylus cantonensis*. Only *Pila scutata* seem likely to be a suitable natural intermediate host for the parasite.

A survey of the food habits of various human communities involved in the eating of these snails is being undertaken and further experimental work with fresh-water snails are still in progress.

Table 1

Direct feeding of naturally infected land molluscs with *Angiostrongylus cantonensis* to four species of fresh-water snails.

Species	Total no. of snails experimented	No. of infected land molluses fed as food for snails	Days snails dissected after last feeding	No. of snails positive for infection	Third-stage larvae			Remarks
					Total	Average per snail	Range	
Pila scutata	50	30	5-10	30 (60%)	1176	59	2-463	Larvae fed to 4 rats. 38 adult worms recovered from two rats died or killed on 15 & 30 days after infection
Bellamyia ingallsiana	50	30	5-10	5 (10%)	23	4	1- 12	Larvae fed to two rats. 10 adult worms were recovered at post mortem.
Brotia costula	50	30	5-10	0	-	-	-	
Tiara sp.	50	30	5-10	0	-	-	-	

Table 2

Infected faeces with *Angiostrongylus cantonensis* obtained from rats and fed to four species of fresh-water snails.

Species	Total no. of snails experimented	Total no. of pieces of infected faeces fed to snails	Days snails dissected after last feeding	No. positive for infection	Third-stage larvae			Remarks
					Total	Average per snail	Range	
Pila scutata	50	50	24-30	2 (4%)	10	5	3-7	Larvae fed to one rat. 3 worms recovered.
Bellamyia ingallsiana	50	50	24-30	0	-	-	-	
Brotia costula	50	50	24-30	0	-	-	-	
Tiara sp.	50	50	24-30	0	-	-	-	

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PRELIMINARY STUDIES ON *ANGIOSTRONGYLUS CANTONENSIS* IN THE MALAYSIAN MONGOOSES

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Attempts of experimental infections with *Angiostrongylus cantonensis* in herbivores and carnivores animals have shown that while some of these animals were not susceptible and others can act as carrier hosts of the parasite (Alicata, 1964 and Weinstein *et al.* 1963). The present study deals with a species of carnivorous mongoose *Herpestes auropunctatus*.

Twenty-one specimens of this mongoose were obtained from areas in northern West Malaysia. The animals were kept in the laboratory and the faeces were collected each morning and checked for parasitic infections for a period of 30 days. Five of the animals died in captivity and the remaining 16 were divided into two groups. Eight animals which were found naturally infected with helminths particularly with a species of metastronglyle nematode, *Pulmonstrongylus herpestis* and two of these were also infected with *Dicrocaelid* trematodes. Remaining 8 animals were all found free of helminth infections. Each animal was artificially infected with 2000 infective stage larvae recovered from naturally infected land slugs (*Microparmarion malayanus*). The control rate were infected with 100 larvae each. The larvae fed to the rats were taken from the same sample that were infected to the mongooses.

Results of the experiments are shown in Table 1&2. In mongooses that were naturally infected with other helminths (Table 1) four groups, two mongooses in each group were artificially infected. Mongooses that were free from helminth infections (Table 2) were also infected. The infected mongooses were killed 7,14,21 and 40 days after they were infected.

In Table 1, only one of the two infected mongooses killed at seven days was found positive with fourth stage worms (two individuals) in the brain. Those animals that were killed at 14;21 and 40 days were all found to be negative. All the control rats that were killed at the same periods were positive for infection.

Table 1
Mongooses exposed to infective stage larve of *Angiostrongylus cantonensis*.

Species	No. of animals exposed	No. of larvae fed	Days animals sacrificed after infection	Pos/ Neg.	No. of larvae recovered			
					Brain	Lungs	Heart	Spinal cord
*Herpestes auropunctatus**	2	2000	7	1/2	2	0	0	0
,,	2	,,	14	0/2	0	0	0	0
,,	2	,,	21	0/2	0	0	0	0
,,	2	,,	40	0/2	0	0	0	0
CONTROLS								
White rats	2	100	7	2/2	55:37	0	0	0
,,	2	,,	14	2/2	41:28	0	0	0
,,	2	,,	21	2/2	37:31	0	0	0
,,	2	,,	40	2/2	0	28:18	5:10	0

* Found naturally infected with a metastrongyle nematode, *Pulmonstrongylus herpestis*, and *Dicrcocaelid* trematodes.

In Table 2, the two infected mongooses that were killed at seven days were both positive with fourth stage worms, (three and four individuals)* in the brain of each of the animals. Two sub-adult worms were recovered from one of the two infected mongooses that were killed at 14 days. In mongooses that were sacrificed after 21 and 40 days, no worms were recovered.

The fourth stage larvae recovered from infected mongooses (Table 1 and 2) were all normal and healthy, except for one worm recovered 14 days after infection, (Table 2) where in one of the two worms recovered in the brain was found dead. The infected mongooses were all very healthy and showed no signs of physical weakness during the period of infections. Autopsy of infected animals that were sacrificed at 7 and 14 days revealed signs of slight dilation of blood vessels in the brains, but in those killed 21 and 40 days, no visible changes were noted.

The results of these experiments indicate that the Malaysian mongooses (*Herpestes auropunctatus*) does not appear to be a suitable host for *Angiostrongylus cantonensis*. Wood (1965) had successfully infected

one Formosan mongoose (*Herpestes urva formosanus*) from which he recovered five adult worms after 75 days of infection. Unless this observation is repeated it will not be possible to say conclusively whether the susceptibility to the Formosan species of mongoose to *Angiostrongylus cantonensis* is different or not.

Such differences in susceptibility have however been demostrated in some of the Malaysian rats which were found naturally resistant to the parasite while other species were susceptible (Heyneman and Lim, 1965). These experiments are preliminary and work on these lines are still in progress.

Table 2
Mongooses exposed to infective larvae of *Angiostrongylus cantonensis*.

Species	No. of animals exposed	No. of larvae fed	Days animals sacrificed after infection	Pos/ Neg.	No. of worms recovered			
					Brain	Lungs	Heart	Spinal cord
*Herpestes auropunctatus**	2	2000	7	2/2	4:3	0	0	0
,,	2	,,	14	1/2	2	0	0	0
,,	2	,,	21	0/2	0	0	0	0
,,	2	,,	40	0/2	0	0	0	0
CONTROLS								
White rats	2	100	7	2/2	44:38	0	0	0
,,	2	,,	14	2/2	55:41	0	0	0
,,	2	,,	21	2/2	40:28	0	0	0
,,	2	,,	40	2/2	0	19:16	12:9	0

* Found free of natural infections with helminths.

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EARLY DEVELOPMENT OF SPOROCYSTS OF *ECHINOSTOMA LINDOENSE* (TREMATODA: ECHINOSTOMATIDAE) IN THE SNAIL *BIOMPHALARIA GLABRATA*†

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Sporocysts of *Echinostoma lindoense*. (Sandground and Bonne) and of various other echinostomes develop in the snail within the ventricular cavity of the heart (Lie 1964; 1968). Their early development in the heart is studied in *Biomphalaria glabrata* snails. The Brazilian strain of *E. lindoense* is used. After penetration into the snail, the young sporocysts migrate to the heart. The migration route is not known. They arrive in the heart cavity within 36 to 40 hours after exposure of the snail to miracidia. Sporocysts do not grow in size during the migration period. Soon after arriving in the ventricular cavity, the anterior end becomes attached to the heart muscle, while the posterior end is free, (Fig. 1 and 2). How

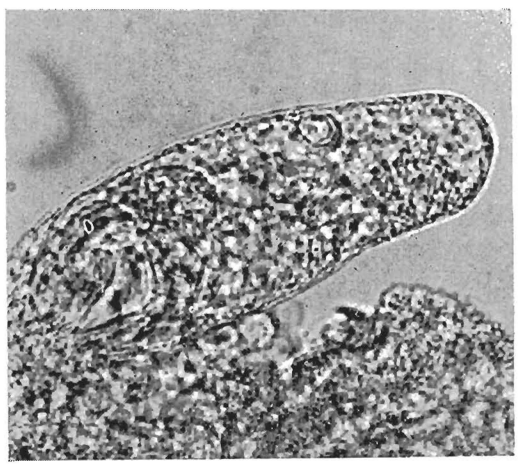

Fig. 1—Sporocyst of *E. lindoense*, 100 × 45 μ, 40 hours after exposure of the snail to miracidia, attached to heart muscle.

†Supported by UC ICMRT with Research Grants TW 00144 and AI 07054, NIH, U.S. Public Health Service.

attachment is accomplished is not known. Rapid growth takes place in the heart cavity. The attached end soon becomes broader than the free end. Within about 4 days after arriving in the heart, the sporocysts are mature. A three day old sporocyst has still one pair of flame cells, just as in the miracidium. The first redia is released 6 days after exposure of the snail to miracidia through a birth pore which is situated close to the place of attachment. The birth pore may be the old opening of the anterior papilla of the miracidium.

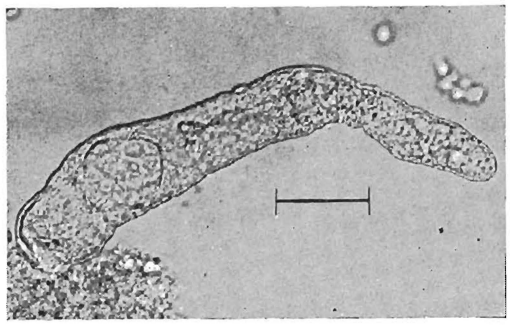

Fig. 2—Sporocyst of *E. lindoense*, 4 days old, attached to heart muscle. The pigment bodies of the eye spot are visible, situated close to the place of attachment.

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DEVELOPING SPOROCYSTS OF *PARYPHOSTOMUM SEGREGATUM* (TREMATODA : ECHINOSTOMATIDAE)†

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Sporocysts of *Paryphostomum segregatum* Dietz develop near the places of entry into the snail (Lie and Basch, 1967), unlike those of various other species which develop in the heart cavity. After penetration into *Biomphalaria glabrata* snails, sporocysts of *P. segregatum* remain under the epithelium, causing small nodules on the surface of the snail. They do not grow in size in the first two days (Fig. 1). A period of adjustment to the new environment is apparently needed before the sporocysts can grow. In the following three days growth is rapid (Fig. 2) and they become mature in 6 days after exposure of the snails to miracidia.

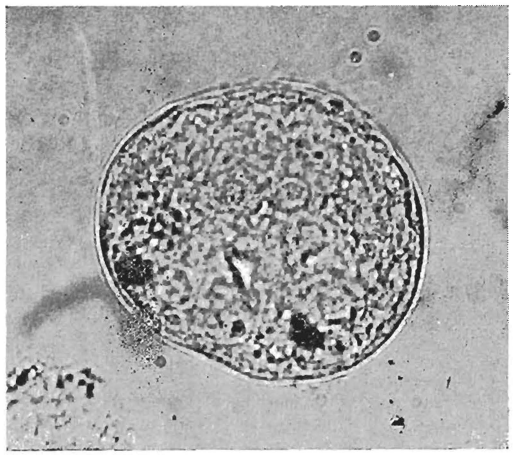

Fig. 1—Sporocyst of *P. segregatum*, 2 days old, dissected from the snail. Note the two large pigmented bodies of the eye spot. Sporocyst 65 × 40 μ, is as large as miracidium.

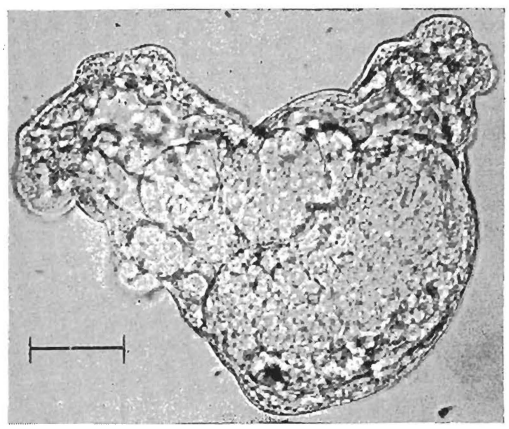

Fig. 2—Sporocyst of *P. segregatum*, 200 × 188 μ, four days old. Scale is for 50 μ.

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† Supported by UC ICMRT, Hooper Foundation, San Francisco School of Medicine with Research Grants TW 00144 and AI 07054, NIH, U.S. Public Health Service.

NOTE ON THE LIFE HISTORY OF *FASCIOLA GIGANTICA* (COBBOLD, 1885) IN WEST MALAYSIA†

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Although Fascioliasis due to *Fasciola gigantica* appears to be a common parasitic disease of cattle in West Malaysia, no complete study of its life history have been undertaken in this country.

Eggs of *F. gigantica* obtained from the gall bladder of infected cattle slaughtered in the Kuala Lumpur Abattoir were washed in three changes of tap water and cultured in rain or river water in small Petri dishes at room temperature (26-28°C). The eggs are operculated, light yellow-brown in colour and measure 137.3 μ to 173.2 μ by 87.8 μ to 103.5 μ (average 146 × 90 μ). The miracidia hatch between 14-17 days. Laboratory bred fresh water snail, *Lymnaea rubiginosa* were exposed to the newly hatched miracidia. Complete penetration takes about 30-40 minutes. The miracidia develop within the snails into sporocyst, which are mainly found in the mantle and in the tissue surrounding the respiratory cavity.

First generation rediae appear in the sporocyst on the 5th day and leave the sporocyst on the 7th-8th day. The newly produced rediae measure about 430 × 120 μ, start to migrate towards the digestive gland (liver) of the snail, feeding on tissue and growing in size. On the 21st-22nd day the rediae reach the size of 1.0-1.5 × 0.2-0.3 mm and usually contain two to five second generation rediae. The second generation rediae leave the mother rediae within 4-5 days and feed on the liver tissue. 26-28 days after infection, the first generation rediae begin to produce cercariae in its body. On the 40th-42nd day,the cercariae start to shed from the snails. Shedding of the cercariae happens only in the dark. For checking infection, the snails were placed in a dark cabinet for one hour. There was no shedding if the snails were exposed to artificial or sunlight.

It has been pointed out by Kendall S.B.(1964) that morphologically identical snails may be receptive in quite different degrees to trematode parasites. Our studies have shown that under the same conditions of exposure, snails from Kuala Pilah ricefield were highly susceptible to *F. gigantica* and usually shed the cerceriae on the 40th day, while those collected from the surroundings of Kuala Lumpur, were poorly susceptible with the cercariae emerging no earlier than 85 days.

Cercariae usually encyst on any vegetation but they also encyst on the glass wall and the host snail shell. Encysted metacercariae were fed to laboratory rabbits, guinea pigs, goat and buffaloes for maintaince of the infection.

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EPIDERMAL PLATES AND PAPILLAE OF MIRACIDIA IN TWO DERMATITIS PRODUCING SCHISTOSOMES IN WEST MALAYSIA†

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Using the silver impregnation technique it is possible to demonstrate the epidermal patterns of the miracidia. Specific identification of the species of schistosome can be based on the number and position of the papillae. Dutt and Srivastava (1961) made a detailed investigation on the miracidia of six species of mammalian schistosomes in India *(Orientobilharzia dattai, Schistosoma indicum, Schistosoma spindale, Schistosoma nasale, Schistosoma incognitum* and *Bivitellobilharzia nairi)* and suggested that this technique could be applied for specific diagnosis of animal Schistosomiasis. Basch (1966) worked out the life cycle of *Trichobilharzia brevis,* which causes dermatitis in Malaysian rice fields. Margono (1968) reported this avian schistosome also as a cause of "Sawah itch" in Djakarta. The papillar structure of the miracidium has not been described in detail. We found that the miracidia of the dermatitis producing schistosomes in West Malaysia, *Trichobilharzia brevis* (Basch, 1966) and *Schistosoma spindale* (Montgomery, 1906) can be easily differentiated by the silver impregnation technique.

Freshly hatched miracidia were obtained from the faeces of experimentally infected ducks and goats. The living miracidia were dropped into hot (about 70°C) 0.5% silver nitrate solution in a test tube or 50 c.c. beakerglass. When settled to the bottom, they were washed in three changes of distilled water

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and then exposed to direct sunlight for ten minutes. They were then washed again in three changes of distilled water and mounted in glycerine for examination. By this technique, the number and the boundaries of the epidermal cells, the number and position of the papillae in the extracellular spaces, the opening of the pentration gland and the excretory pore become sharply delineated.

Trichobilharzia brevis. The miracidium possesses 22 epidermal cells arranged in four tiers of 6, 9, 4 and 3 cells respectively. No variation in the number of cells was met in the study of 30 miracidia (Fig. 1).

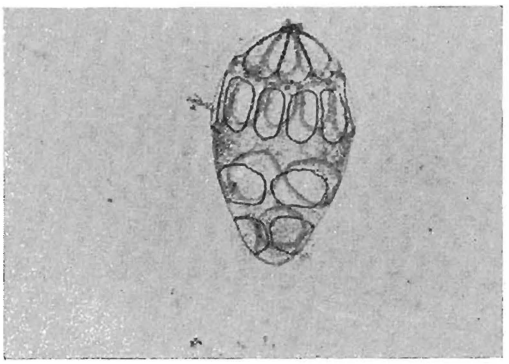

Fig. 1—*Trichobilharzia brevis.* The miracidium with 22 epidermal cells arranged in 4 tiers of 6, 9, 4 and 3 cells respectively.

Five sets of papillae-like structures are situated in the extracellular spaces. The first set consists of 2 groups of papillae situated at the base of the tenebratorium. Each group consists of 9 papillae, three large and six small papillae. The former are the openings of the penetration and apical glands; the latter appear to be sensory in nature and constitute the first group of bristle patches. The second set consists of six small papillae, each of which is situated externally to the middle of the base of each epidermal cell of the first tier. The third set consists of four papillae, two at each laternal aspect of the miracidium, situated in the space between the first and second tiers of epidermal cells. The fourth set consists of 12 papillae, each of which is situated external to the side of the base of each epidermal cell of the first tier. They are the second group of bristle patches, and form a girdle around this region (Fig. 2). The fifth set consists of two small papillae situated in the intercellular space between the dorsal and ventral epidermal cells of the third tier.

Schistosoma spindale. The miracidium of *S. spindale* also possesses 22 epidermal cells arranged in four tiers of 6, 9, 4 and 3 cells respectively. No variation in number was noticed in the study of 30 miracidia (Fig. 3). They posses six sets of papillae. The first set and the second set are similar to those of *T. brevis* in number and position. The third set consists of 2 papillae only, one at each lateral aspect of the miracidium. The fourth set consists of six papillae,

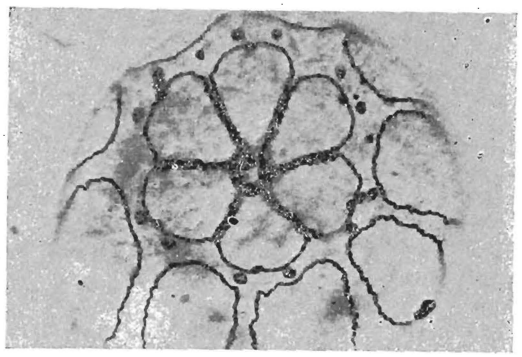

Fig. 2—*Trichobilharzia brevis* (see text) group of bristle patches.

three on each side. Of these, one is located dorsally, one laterally and one ventrally. They are the second bristle patches. The fifth set consists of two small papillae situated one on each lateral side between second and third tiers of cells. The sixth set consists of 20-22 larger papillae situated also in the space between the second and third tiers of cells. They are the third bristle patches and forms a girdle around this region. Out of the 30 miracidia studied, 6 had 22 papillae in this set.

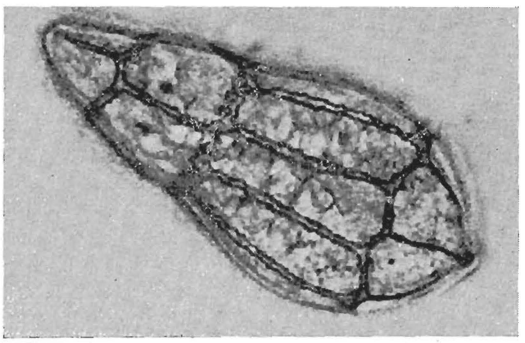

Fig. 3—The miracidium of *Schistosoma spindale.*

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TRANSMISSION OF *PLASMODIUM CYNOMOLGI* (PERLIS STRAIN) TO MAN

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Eyles *et al.*, (1960) reported the transmission of *P. cynomolgi* to man by mosquito bite. Since then several other strains of *P. cynomolgi* have been transmitted to man by mosquito bite, "M" strain of *P. cynomolgi* (Coatney *et al.* 1961, Schmidt 1961); a strain of *P. cynomolgi* isolated from *Macaca nemestrina* in Malaya (Contacos and Coatney 1963) and a strain isolated from *Macaca irus* in Cambodia (Bennett and Warren 1963). The present note reports the transmission of *P. cynomolgi* isolated from *A. balabacensis* in North Perlis, North Malaya to man.

Source of Parasite

In December 1964 and January 1965 field studies of human and simian malaria were conducted in the monsoon forests, the State of Perlis, in the Northern Malay States. The relative attraction of man and monkeys for anophelines in the area was determined by trapping mosquitoes in monkey baited traps on platforms in the forest and in human-baited net traps on the ground. All *A. balabacensis* caught were dissected and the salivary glands examined for sporozoites, which were inoculated intravenously into malaria-free rhesus monkeys. *P. inui* and *P. cynomolgi* infections were isolated (Cheong *et al.* 1965). The *P. cynomolgi* strain was lost when the monkey died. In December 1966 a second isolation was made of *P. cynomolgi* in the same area.

Experimental Transmission

Laboratory bred *A. balabacensis* were fed on 26th March 1969 on a rhesus monkey which was infected with *P. cynomolgi* isolated from *A. balabacensis* in North Perlis. At 6.5 days after feeding, the midguts were positive with oocysts and at 10.5 days the salivary glands were found heavily infected with sporozoites. On 12th April 1969, 10 mosquitoes were allowed to re-feed on a volunteer (G.L.C.) 16.5 days after the mosquitoes had fed on the monkey, of which 4 fed to engorgement. They were dissected twenty minutes after they were fed and the salivary glands were found positive with sporozoites.

On 22nd April 1969 the volunteer had a headache and slight chill. Blood films taken on the same day were negative, then on 25th April at 7 p.m. the volunteer had a severe chill and with a temperature of 99°F. Blood films taken on the next day were positive with *vivax* like parasites, 3 parasites were found in three blood films examined. 4.5 ml of blood from the volunteer was inoculated intravenously to a rhesus monkey which demonstrated *P. cynomolgi* parasiest twelve days later. Blood films taken from the volunteer on 28th, 29th, 30th April and on the 2nd May were positive with typical vivax-like parasites. No severe clinical symptoms was observed by the volunteer.

On 3rd May 1969 the infection was terminated with chloroquine. *P. cynomolgi* from Perlis in monkey and man are demonstrated.

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A NEW BIRD FILARIOD ISOLATED FROM *MANSONIA CRASSIPES* IN MALAYSIA

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Avian filariods have been poorly studied in Malaysia. The natural vectors of those which are already known have been even more poorly studied. In 1964, during a survey for *Plasmodium juxtanucleare* in chickens, some blood films showed microfilariae of an unknown species (Bennett *et al.* 1966). In 1965 the developing stages of two types of bird filariods were reported from *Mansonia crassipes* by Niles, Fernando and Dissanaike in Ceylon. One has been described as *Cardio filaria nilesi*, a new species (Dissanaike and Fernando 1965) and the other as *Pelecitus ceylonensis*, another new species (Dissanaike 1967). *Cardiofilaria nilesi* was experimentally isolated from chicks and what appears to be the same species was also naturally found in other birds such as crows, mynahs, barbets, wild fowls and herons. *Pelecitus ceylonensis* was isolated from chicks and Ash-doves and was also naturally found in crows and possibly mynahs.

In our studies carried out for the incrimination of vectors of simian malaria in a swamp forest area near the coast of Selangor State all mosquitoes trapped by human or monkey platform baits were dissected for possible infection with parasites. *Mansonia crassipes* a member of the *Coquilettidia* group was on many occasions found with unknown filaria infective larvae as well as with oocysts and sporozoites of unknown malaria species possibly *Plasmodium traguli* (Warren *et al.* 1964) from the Mouse deer (*Tragulus javanicus*) or of an avian species. The mosquitoes were also noted to harbour a certain *Crithidia* species. Members of the subgenus *Crithidia* have been known to show a

strong predilection for bird blood (Williams *et al.* 1958; Wharton, 1962, Niles, 1963).

Towards the end of August 1968 a single *Mansonia crassipes* attracted to a monkey bait caught and dissected, was found to harbour 31 infective larvae of an unknown filariod. Twenty-six of these larvae were inoculated into a week old chick (001) intramuscularly for further observations. 77 days after inoculation of the infective larvae blood films obtained from the chick were found positive for microfilariae.

This demonstration shows of the preliminary observations on the parasite. No specific identification of the parasite had yet been made.

Measurements of three infective larvae studied were as follows:—

	Length	Max. width	Anal ratio	Nerve ring	Terminal papillae
Specimen 1	1,650μ	27.2μ	2.8	109μ	3 distinct
Specimen 2	1,675μ	23.8μ	4.7	115μ	-
Specimen 3	1,875μ	27.2μ	3.3	119μ	3 distinct

Measurements were also made on 10 microfilariae and these were:—

	Length	Cephalic space	Breadth	Inner body
1.	275μ	-	5μ	60μ
2.	325μ	6μ	5μ	72μ
3.	270μ	9μ	5μ	57μ
4.	270μ	5μ	4μ	60μ
5.	260μ	7μ	4μ	60μ
6.	270μ	7μ	5μ	47μ
7.	295μ	5μ	5μ	65μ
8.	265μ	7μ	5μ	58μ
9.	240μ	7μ	5μ	52μ
10.	284μ	7μ	5μ	-
Average:	275μ	6.7μ	4.8μ	59μ

From the above preliminary study, the measurements appear to tally fairly well with those of *Cardiofilaria nilesi* in so far as the infective larvae are concerned. The total length falls within range and so also the nerve ring. The three distinct terminal papillae also appear similar but the anal ratio however seem to be higher than in *C. nilesi*. Development takes place in the thoracic muscles of the mosquitoes. The length of the microfilariae appears shorter than the length given for *C. nilesi*. The average length in the present study was 275μ whereas in *C. nilesi* it was reported to be 350μ (Dissanaike & Fernando 1965). However, (Niles *et al.* 1965) reported the length of the microfilariae to be in the region of 280-300μ. The ratio of the cephalic space to the width of the microfilariae in the present study is about $1\frac{1}{2}$:1. The microfilariae are unsheathed and without a terminal nuclei. It is unlike *Pelecitus ceylonensis*.

Mosquito feedings were carried out on the chick using several species ofculicines and anophelines but only three species did support development of the larvae. These were *M. annulata* (11% infection rate) and *Aedes togoi* (59% infection rate) both species producing very few infective larvae. The natural vector *M. crassipes* when fed however, gave a very high infection rate (97%) and produced a large number of infective larvae.

The microfilariae, infective larvae obtained from experimental mosquitoes, as well as the natural host mosquito along with results of feeding were demonstrated.

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SUSCEPTIBILITY STUDIES ON THE HOUSE FLY, *MUSCA DOMESTICA VICINA L.*†

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In 1962 during studies on flies from Cameron Highlands, Malaysia, Wharton *et al.* (1962) carried out simple insecticide tests on flies obtained from the highlands as well as those obtained from the lowlands. These studies were based on knock down rates. Four insecticides were tested and these were D.D.T., B.H.C., malathion and diazinon. There were indications that the flies both from the highlands and lowlands were resistant to D.D.T. and B.H.C.

In September 1968 J. Kieding, a W.H.O. consultant at the request of the Institute for Medical Research, Kuala Lumpur and Ministry of Health, Malaysia spent a month studying the fly problem at the Highlands.

Six insecticides were screened against the house fly, *Musca domestica vicina* caught from two different localities (i) highland flies from Cameron Highlands (Tringkap and Mensum Farms) and (ii) lowland flies from the vicinity of Kuala Lumpur (Ampang village). The insecticides represented 2 hydrocarbons, D.D.T. and Lindane (B.H.C.) and 4 organo-phosphorus compounds, Dimethoate, Fenthion, Diazinon and Diptrex.

In all the tests flies were given topical application of E.M.K. (Ethyl Methyl Ketone) (W.H.O. technique) solutions of technical grade insecticides of suitable concentrations so as to give a range of mortality for each batch of flies. The insecticides were applied to the dorsum of the thorax of the fly by using a standard drop pipette calibrated to deliver a drop size of 0.38 micro litre. Flies were immobilized with ice before treatment by putting them at 1-2 °C for about 10 minutes. The immobilized flies after treatment were placed in paper cups lined at the bottom with paper foil. A cube of sugar and moist lint were placed on the net cover of the cups. Twenty flies were treated with each concentration and the number dead after 24 hours were recorded. Only female flies were tested. Corrections for the occasional natural mortality observed in control samples were made by Abbott's formula. Tests were carried out at room temperature (range 28°-31°C).

† This work was started by J. Kieding, W.H.O. consultant during his month's stay here and the results are mainly drawn from his and some of our results.

RESULTS

Of the two hydrocarbon compounds tested Lindane at 10.24% gave a 100% kill of lowland flies and 40% kill of highland flies, while D.D.T. at the same concentration gave 30% kill and 20% kill of lowland and highland flies, respectively. Organo-phosphorus compounds that gave a 100% kill below 1% concentration of the insecticide were Dimethoate, Fenthion and Diazinon (in the order of effectiveness). Diptrex at a concentration of 1.28% gave a 92% kill of lowland flies and 15.0% kill of highland flies.

From the results obtained, the lowland flies were more susceptible to the whole range of the six insecticides tested; presumably this could be due to the fact that the highland flies were on the average heavier in weight (mean = 17.5 mg) than the lowland flies (mean = 14.3 mg). However, the range in weight of the lowland flies was greater 3 mg. to 36 mg. as against 5 mg. to 32 mg. in highland flies. The kills for the highland flies were almost always lower than for the lowland flies at similar concentrations.

Conclusions

1. Highland flies are more consistant in weight than lowland flies.
2. Highland flies are less susceptible to all the insecticides than lowland flies.
3. Highland flies are clearly resistant to Lindane, D.D.T. and Diptrex and fairly susceptible to Dimethoate, Fenthion and Diazinon.
4. The lowland flies are resistant to D.D.T. fairly susceptible to Lindane and susceptible to Dimethoate, Fenthion and Diazinon.

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THE SPOROGONIC CYCLE OF *P. CYNOMOLGI BASTIANELLII* and *P. CYNOMOLGI* BEROK STRAIN IN *A. BALABACENSIS BALABACENSIS*

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The sporogonic development of five different strains of *Plasmodium cynomolgi*, (Cambodia, Ulu Gombak, Mulligan, Bastianellii and Berok) in a few species of Anopheles have been reported by Bennett *et al.* (1966 a, b.). Warren *et al.* (1963) has also reported on the susceptibility of some species to *P. cynomolgi bastianellii* to *Anopheles* mosquitoes. Cheong *et al.* (1969) reported on the sporogonic cycle of *P. cynomolgi* obtained from Perlis Malaysia isolated from *A. b. balabacensis* in four species of Anophelines. The susceptibility of *A. b. balabacensis* an important human malaria vector, has however not been studied for *P. cynomolgi bastianellii* and *P. cynomolgi* Berok strains.

The following is a note on the sporogonic development of the above two strains in *A. b. balabacensis.*

P. CYNOMOLGI BASTIANELLII

Midguts were examined for the presence of oocysts at 3.5 days after feeding on an infected rhesus monkey. The oocysts measured 14.1μ in diameter with a central clump of pigment and were spherical in shape. At 6.5 days the oocysts averaged 42.5μ in diameter with dispersed pigments. The size of the oocysts increased in diameter and on 7.5 days the average was 49.0μ. The largest oocysts measured was 78.0μ on day 7.5. Differentiated as well as degenerate oocysts were noted from 6.5 onwards. The numbers of degenerated oocysts increased with time to a maximum of about 25% of the oocysts being affected. Sporozoites were seen in salivary glands 8.5 days one day earlier than reported for this parasite in other species of mosquitoes. In *A. maculatus* degenerated oocysts usually appeared from 9.5 days onwards when 25% of specimens examined had sporozoites seen in their glands. In *A. balabacensis* when sporozoites were first noted 100% of the glands examined were positive for each consecutive day subsequently. The average number of oocysts per gut seem to be more in *A. b. balabacensis* compared to other species however, the sizes of the oocysts appear smaller compared to 78μ in diameter to 90μ in *A. maculatus* and 98μ in *A. kochi.* In *A. maculatus* the average diameter was 55μ compared to 49.0μ *A. b. balabacensis.*

P. CYNOMOLGI BEROK

Similar observations were made for the Berok strain *A. b. balabacensis.* At 3.5 days the oocysts measured 14.1μ in diameter with a central clump of pigment and at 6.5 days, the pigments dispersed and oocysts measured 39.6μ in diameter. At 8.5 days the largest oocysts were observed measuring 57.2μ. Differentiation of oocysts began at 5.5 days together with presence of degenerated oocysts. Sporozoites were seen on the 7.5 day, one day earlier than has been reported for this parasite in other species of mosquitoes. There are differences again in the sizes of mature oocysts as well as largest oocyst size noted when compared to *A. maculatus.* Here again, the mature oocysts had an average size slightly smaller than those for *A. maculatus* but the maximum oocyst size was much smaller i.e. 57.2μ to 75μ. When compared with maximum oocyst size for *P. cynomolgi bastianellii,* and *P. cynomolgi* Perlis strains, the Berok strain produces the smallest. The day of sporozoite appearance in the glands was however the earliest. The average number of oocysts per gut was more than in *A. maculatus* but degeneration of oocysts takes place earlier than in *A. maculatus* for the same strain. The infectivity of the parasite to *A. b. balabacensis* was very high as in the case of *P. cynomolgi bastianellii.*

A. b. balabacensis is thus shown to be an efficient host for *P. cynomolgi bastianellii* and for *P. cynomolgi* Berok strains, althought he maximum size of the. oocysts were not as large as in other species seen The sporozoites appeared in each case one day earlier than has been reported for other species. This earlier development of sporozoites has also been shown for the Perlis strain of *P. cynomolgi.*

The accompanying Tables summarises our observations.

A. BALABACENSIS BALABACENSIS FED ON *P. CYNOMOLGI BASTIANELLII*

	Days after Feeding							
	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5
Number of mosquitoes examined	2	3	3	3	3	3	3	3
Average number of oocysts	152	271	305	308	179	88	83	57
Minimum size of oocysts	13.0μ	15.6	23.4	33.8	36.4	31.2	31.2	33.8
Maximum size of oocysts	15.6μ	23.4	46.8	54.6	78.0	62.4	39.8	62.4
Average size of oocysts	14.1μ	18.9	38.6	42.5	49.5	46.7	42.9	49.0
Normal oocysts %				96	96	81	76	83
Degenerated oocysts	0	0	0	4%	4%	19%	24%	17%
Day when sporozoites were first seen				Neg.	Neg.	+	+	+

A. BALABACENSIS BALABACENSIS FED ON *P. CYNOMOLGI* BEROK STRAIN

	Days after Feeding						
	3.5	4.5	5.5	6.5	7.5	8.5	9.5
Number of mosquitoes examined	3	2	2	2	2	2	2
Average number of oocysts	339	275	181	108	128	57	79
Minimum size of oocysts	10.4	18.2	18.2	31.2	26.0	31.2	39.0
Maximum size of oocysts	15.6	23.4	31.2	49.4	46.8	57.2	52.0
Average size of oocysts	14.1	20.7	27.1	39.6	39.2	47.1	45.0
Normal oocysts %	100	100	100	96	93	91	90
Degenerated oocysts %	0	0	4%	4%	7%	9%	10%
Day when sporozoites were first seen				Neg.	+	+	+

SPOROGONIC DEVELOPMENT OF *P. CYNOMOLGI* SPP.

Species	Maximum size oocysts	Day sporozoites first seen
P. cynomolgi bastianellii		
A. maculatus	90μ	9.5 days
A. kochi	98μ	9.5 ,,
A. balabacensis	78μ	8.5 ,,
P. cynomolgi Berok		
A. maculatus	75μ	8.5 days
A. balabacensis	57μ	7.5 ,,
P. cynomolgi Perlis		
A. maculatus	70.2μ	Negative
A. kochi	98.8μ	9.5 days
A. balabacensis	98.8μ	8.5 ,,
A. sundaicus	72.0μ	9.5 ,,

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THE EFFECT OF SLOW DESICCATION OF *ANOPHELES HACKERI* EGGS ON LARVAL HATCHING

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In a previous note (Cheong and Sta Maria 1969), it was shown that the eggs of *A. balabacensis balabacensis*, a member of the 'leucosphyrus' group and an important vector of human and simian malarias in Malaysia were able to withstand a period of slow desiccation of up to two weeks duration or more. It has been pointed out that this is of great advantage to the species survival, because its breeding grounds are of temporary nature and often become scarce when the dry season approaches.

The following is a note on the effect of the slow drying process on the hatching ability of the eggs of *Anopheles hackeri* another member of the 'leucosphyrus' group and an important natural vector of five species of simian malarias along the Selangor coast, in W. Malaysia. It has been reported elsewhere (Cheong and Mahadevan 1967) that this species is unique in its breeding habits in being found only in collections of water at the cut bases of the nipah palm *(Nipah fruticans)* leaves which are cultivated. In a previous note, (Cheong and Mahadevan 1969), we have also commented upon the small numbers of *A. hackeri* larvae usually found in their breeding holes and showed that one of the factors for this reduction in numbers is the presence of at least four forms of predators namely, dragonfly larvae, damsel fly larvae, *Toxorhynchites* larvae and diving beetles, in the same environment.

We were able to demonstrate in the laboratory now another reason for the small numbers of larvae seen. Eggs collected from wild caught gravid females were put out to hatch at daily intervals after being slowly dried in a moist-chamber. The preliminary results shown in the Table indicate that the eggs could withstand quite a long period of slow drying of up to eleven days when 10% still hatched. This is of course five days or more short of the period *A. balabacensis balabacensis* is able to withstand desiccation.

Delayed Hatching in *A. hackeri* Eggs.

No. of days in moist chamber	No. of eggs exposed	No. hatched	% hatched
1	232	189	81.4%
2	207	151	72.9%
3	475	250	52.6%
4	279	155	55.5%
5	515	123	23.8%
6	485	134	27.6%
7	244	45	18.4%
8	465	49	10.5%
9	279	7	3.0%
10	225	6	2.7%
11	100	10	10.0%
12	163	0	0
13	130	0	0
14	-	-	-
15	285	0	0

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THE SEARCH FOR ATTACHED EGGS OF *ANOPHELES HACKERI* ON NIPAH BASES:- ANOTHER FACTOR IN THE SMALL NUMBERS OF LARVAE BEING OBSERVED IN NATURE

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Several reports have been made on various aspects of ecology of *A. hackeri* recently(Cheong-unpublished, Scanlon *et al.* 1968, Cheong and Mahadevan, 1967, Cheong and Mahadevan 1968, Cheong and Mahadevan 1970).

Investigations towards the reasons for the peculiarity in finding only small numbers of *A. hackeri* larvae in nature were carried out. Two of the factors which were determined earlier were (1) the fact that there were several species of insects which have been predating on the larvae and (2) the fact that *A. hackeri* eggs could withstand a certain amount of desiccation for up to eleven days before hatching. Together with these, we have now found that there is evidence of (a) some eggs are being laid attached to the sides of cut nipah plant stems and possibly these eggs are eaten by predators like mites or perhaps washed up by the water and left to dry (b) the delayed hatching phenomemon.

The following is a note on the results of scrappings done on the base of the nipah palms. The results are tabulated and demonstrated. Two forms of leaf bases were scrapped, one with a smooth internal surface and the other with a rough decayed internal surface. A total of 358 scrappings were made. The axils were classified into three forms (a) those with water and with *A. hackeri* larvae present (b) those with water but without *A. hackeri* larvae present and (c) those without any water (dry). In every type of situation whether it was from the smooth or rough surfaces, from axils with or without larvae or with or without water, *A. hackeri* larvae were found to hatch out when the scrappings were placed in bowls of water and observed over a period of a few days. Hatchings from dry surfaces produced the lowest figures 11 (3.1%) while those areas which had *A. hackeri* larvae in them were positive 29 times (8.1%). Those which had water but no *A. hackeri* larvae were positive 20 times (5.6%). A total of 60 positives were found in 358 scrappings made (16.8%).

It was observed that the appearance of the larvae in the bowls was at irregular intervals in most instances, varying from one day to several days and this explains the reason for the larvae in nature to be so often in different stages of development. This phenomenon of delayed hatching has been observed for other species of anophelines.

27 of the scrappings were positive for other insects. Two were positive for dragon fly and damsel flies and twenty-five were positive for *Aedes butleri*, *Aedes fumidus*, *Aedes bonneae*, and *Aedes musculinus*, *Toxorhynchites splendens*, *Uranotaenia lateralis* and *Culex* (*Lophos.*), *Culex fragilis* and *Culex obscurus*.

Internal Scrappings of Cut Nipah Axils for *A. hackeri* Eggs.

Type of Stem Surface	Number Scrapped	Scrapping Positive for *A. hackeri* Eggs			
		Axils containing *A. hackeri* larvae	Axils containing no *A. hackeri* larvae	Dry axils	Total
Smooth Internal Surface	229	18(7.9%)	16(7.0%)	6(2.6%)	40(17.5%)
Rough Decayed Surface	129	11(8.5%)	4(3.1%)	5(3.9%)	20(15.5%)
Total	358	29(8.1%)	20(5.6%)	11(3.1%)	60(16.8%)

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IMMUNOFLUORESCENT STUDIES ON MOSQUITO SPERMS

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Antigenic relationship between various species of mosquitoes have been studied by us using the gel-diffusion technique (Zaman & Chellappah, 1965). As an extension to this work we have now conducted immunofluorescent studies on mosquito sperms. To the best of our knowledge this technique has not been applied previously to mosquito sperms.

Culex fatigans sperms were obtained from female mosquitoes, approximately 3 days after mating. Spermathecae were dissected and placed in a cavity block containing a few drops of sterile physiological saline. The spermathecae were then punctured under a dissecting microscope and actively moving sperms were removed with a pasteur pipette into another cavity block containing physiological saline. Sperms collected from about 20 female mosquitoes were pooled and injected intravenously into 2 rabbits. Each rabbit received approximately 8 to 10 such injections given at weekly intervals. Sera were collected 10 days after the last injection and the globulin fraction was conjugated with fluorescent isothiocyanate, according to the method of Rinderknecht (1960). The unconjugated dye was separated by passing it through a column of Sephadex G-25 and the serum was absorbed against rabbit liver powder before use.

The antigens were prepared by dropping a heavy suspension of sperms on the centre of microscope slides. These were allowed to dry in a dessicator at 5°C for 24 hours. The fixed cells were then treated with 1:5 dilutions of specific antiserum for half an hour at room temperature, washed in phosphate buffered saline and examined under Zeiss fluorescent microscope.

The controls consisted of cells identically prepared but treated with normal conjugated rabbit serum. In addition some slides were pretreated with unconjugated antiserum and then with conjugated antiserum to test for the inhibition of the reaction.

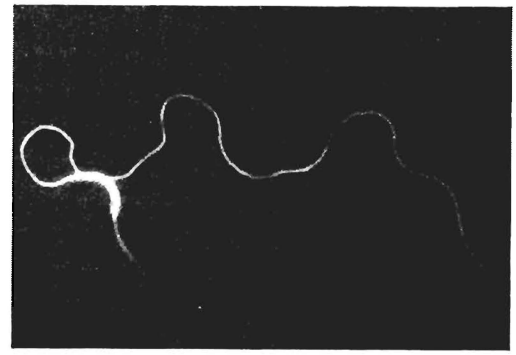

Fig. 1—A mosquito sperm after treatment with fluorescein conjugated antiserum. The fluorescence is particularly marked in the middle piece.

The test slide showed intense fluorescence of sperms. The head, the middle piece and the tail region were clearly visible. The fluorescence was particularly bright in the region of the middle piece. There was negligible fluorescence in the controls and the reaction was inhibited by pretreatment with unconjugated antiserum. Further work is now in progress to study the antigenic relationships of mosquito sperms of different genera and species using this technique.

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CHARCOT-LEYDEN CRYSTALS IN A CASE OF HYPEREOSINOPHILIA

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Charcot-leyden crystals are commonly found in the faeces and sputum in various intestinal and pulmonary parasitic infections. It is now known that these are essentially derived from eosinophils and a considerable amount of work has been done recently on their genesis, ultra structure and biochemical characters (Beaver, 1962; Hornung, 1962; Markert, 1966).

In the light microscope they appear as two hexagonal pyramids placed base to base. They vary in size and may extend from 5-100μ in length, the average being 20-40μ.

In addition to the faeces and sputum they have been found in tissue exudates when there is a heavy infiltration with eosinophils. Beaver and Danaraj (1958) have described their presence in a case of pulmonary ascariasis.

In this particular case the patient had a high eosiinophilic count for more than two years and impr esson

smears made from a lymph gland showed a large number of crystals both in the fresh exudate and also in the Giemsa stained preparation (Fig. 1). Tissue sections of the same gland, however, did not show the crystals. It would seem that a useful method of demonstrating the crystals in tissues would be to make impression smears from fresh biopsy material, whenever this is possible.

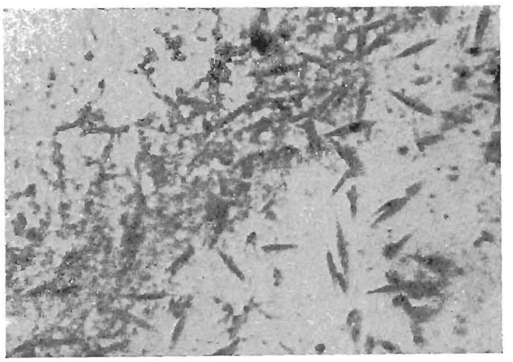

Fig. 1—Impression smear from a lymph gland showing a large number of charcotleyden crystals.

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DISTRIBUTION OF AMOEBIC DYSENTERY COMPARED TO TUBERCULOSIS AND SALMONELLA IN THE ORANG ASLI (ABORIGINE)

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All cases of *E. histolytica*, tuberculosis and *Salmonella typhi* diagnosed at the Gombak Aborigine Hospital near Kuala Lumpur were analysed for geographical distribution. Villages were divided geographically into those located either in fringe and lowland jungle or highland deep jungle. Prevalence figures for the year 1966-1968 for *E. histolytica*, tuberculosis and *typhoid* in these two distinct geographical areas are presented below.

	E. histolytica	Tuberculosis	Typhoid
Total number of cases	180	189	16
Fringe and lowland jungle	176	117	9
Highland primary jungle	4	72	7

From these figures it can be seen that both tuberculosis and typhoid are relatively well distributed through deep jungle. In contrast Amoebic disease is extremely rare in this area and for all practical purposes is confined to fringe and lowland jungle villages.

The marked contrast in the number of cases of *E. histolytica* originating in fringe lowland jungle when compared to highland primary jungle suggest interesting future sero-epidemiological studies.

THE HISTOPATHOLOGY OF HYDROPS FOETALIS DUE TO α-THALASSAEMIA†

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Since the first cases described in 1960-1962 by one of us (Lie-Injo) from Indonesia and Malaya, many other cases have been found in Malaya, Singapore, Thailand, Hongkong and Taiwan, and they clearly show that abnormal haemoglobin production and not iso-immunisation is the major cause of hydrops foetalis in Chinese and in Thais. Although clinical and haematological findings have been described in many cases, the histopathology has little been studied. We are presenting the findings in 34 cases.

The most important and constant histological findings are excessive extramedullary erythropoesis and oedema in various organs. Persistence of Langhans' cells and prominent Hofbauer cells in the placenta, enlargement of the islets of Langerhans in the pancreas and enlargement and changes in the adrenal glands. Sickling of erythrocytes are especially seen in the spleen, bone marrow and in many clogged blood vessels. Unusual aggregates of sudanophilic cells in the interstital tissue of the lung, myocardium and placenta were observed in several cases.

Slides under the microscopes and photographs demonstrate the typical changes in the blood, placenta, liver, spleen, bone marrow, blood vessels, lung, heart, kidney, brain, pancreas, intestine and adrenals.

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