The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. J. W. FIELD
(Malacca Agricultural Medical Board),

DR. A. A. SANDOSHAM and MR. YAP LOY FONG
(Division of Malaria and Filariasis IMR, Kuala Lumpur)

A simplified rapid method of Romanowsky staining for thin blood films

The method has been briefly described in the *Trans. R. Soc. trop. Med. Hyg.*, 1963, *57* (6), 487. Fix the thin film with 10 drops of 0.2% eosin Y in methanol (Analar), then immediately add 20 drops of Field's Stain 'A' (with the addition of two drops of 40% w/v cetrimide B.P. ('Cetavlon' Concentrate, I.C.I.) to 60 ml of stain in dropping bottle), *agitate* to mix and stain for 2 seconds to 5 minutes according to preference: *flush* off the stain for 2 seconds with water, and place the slide on end to drain and dry.

The demonstration slides showed thin blood films with *Plasmodium cynomolgi bastianellii* stained for 2 seconds, 30 seconds, and 3 minutes, respectively. The nuclear chromatin and cytoplasm of the parasites and the stippling in the host cells were well defined after 2 seconds' staining: at 3 minutes the uninfected erythrocytes were very pale, with the infected cells and stippling prominently stained and in sharp colour contrast.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. McWILSON WARREN, MR. KADIR ALI and
DR. GORDON F. BENNETT
(U.S.P.H.S., Far East Research Project, IMR, Kuala Lumpur)
and
PROF. A. A. SANDOSHAM
(Division of Malaria and Filariasis, IMR, Kuala Lumpur)

Morphology of **Plasmodium fieldi** in different species of the genus **Macaca**

Plasmodium fieldi was originally described from *Macaca nemestrina* from Malaya and the morphological features which give this parasite specific status are quite distinct. However, recent observations in this laboratory have shown that there are consistant differences in the morphology of peripheral blood stages of *P. fieldi* in various members of the genus *Macaca*. Eyles et al. (1962) studied one strain of this species of *Plasmodium* in *Macaca mulatta* and noted that the coalescence of the eosinophilic inclusion bodies was much less frequently seen in this monkey than in *Macaca nemestrina*, where this striking characteristic is quite diagnostic. We have now observed this parasite in a number of *M. mulatta*, *M. nemestrina* and *M. irus* and with a moderately infected blood smear, which is well stained, feel reasonably confident not only of a species diagnosis of the parasite but of the host animal as well.

Briefly reviewed, the appearance of *P. fieldi* in the peripheral blood of the above mentioned three species of macaques is as follows.

Macaca nemestrina: Trophozoites — cytoplasm compact; vacuole frequently large; stippling of the Schuffner type but abundant and coarse, tending to coalesce into large eosinophilic masses in older forms; pigment evently distributed; infected cell not enlarged or only slightly enlarged. Schizonts averaging 12 merozoites when mature; inclusion bodies oriented peripherally to form an intense eosinophilic ring around the parasite.

Macaca irus: Trophozoites — cytoplasm compact but not so dense as that seen in *Macaca nemestrina;* vacuole large; stippling of the Schuffner type but less abundant than in *M. nemestrina* — does not coalesce into large masses, cell enlargement marked. Schizonts as in *M. nemestrina* except that the inclusion bodies remain coarsely granular and the eosinophilic ring is much less intense.

Macaca mulatta: Trophozoites — cytoplasm compact; vacuole large; stippling more coarse than that of *P. cynomolgi* but does not form large masses; more cell enlargement than in *M. nemestrina* but less than in *M. irus*. Schizonts as in *M. irus*.

Comment: It is sometimes difficult to separate *P. fieldi* from *P. cynomolgi* in *M. irus* and *M. mulatta*. However, the compactness of the cytoplasm, the relative lack of amoeboidicity and the quite coarse nature of the inclusion bodies makes specific identification easy in moderate infections. The very light natural infections in *M. irus* are more difficult and frequently impossible to separate from *P. cynomolgi*. However, this parasite is quickly identified in *M. nemestrina* and in experimental infections in *M. mulatta* produce a much less intense parasitaemia than *P. cynomolgi*.

Demonstrations showed Giemsa-stained thin blood films from *Macca irus*, *M. mulatta* and *M. nemestrina* infected with *P. fieldi*. The morphological variations mentioned here are seen in these specimens and in black and white sketches.

REFERENCE

Eyles, Don E., A. B. G. Laing and Yap Loy Fong, 1962. **Plasmodium fieldi** sp. nov., a new species of malaria parasite from the pig-tailed macaque in Malaya. **Ann. trop. Med. Parasit. 56,** 242-247.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. GORDON F. BENNETT and DR. McWILSON WARREN
(U.S.P.H.S., Far East Research Project, IMR, Kuala Lumpur)

and

MR. W. H. CHEONG
(Division of Entomology, IMR, Kuala Lumpur)

Variants of **Plasmodium cynomolgi** identified by characteristics of the sporogonic cycles

The identification of morphologically indistinguishable variants of a *Plasmodium* has always been arduous and time consuming. Any means by which identification could be simplified would be of great advantage. Usually, identification of strains has been based on differences appearing during the schizogonic cycle in the vertebrate host. Relatively little attention has been paid to differences in the sporogonic cycle, although a number of potentially useful characteristics occur.

Recently, two new strains of *Plasmodium cynomolgi* were isolated by this laboratory from west-central Cambodia and the Gombak region near Kuala Lumpur. Initial studies on the schizogonic cycle indicated that both new strains differed from the previously known *cynomolgi* parasite from Malaya. However, these differences did not become apparent until the course of infection in several animals had been studied for 30 days or more. In addition, the two new strains could not be separated from each other on the basis of differences in the schizogonic cycle.

Studies on the sporogonic cycle of the three strains *P. cynomolgi* established that differences occurred in (1) the rate of development of the cycle, (2) the size of the mature oocysts and (3) the susceptibility of different Malayan anophelines. The rate of sporogony differed sharply between the Cambodian strain (completed in 7.5 days) and the other cynomolgis (completed in 9.5 days). Oocysts of the former parasite consistently averaged about one-third smaller than those of the latter. Marked differences were also noted between the susceptibility of various Malayan anophelines to these malarias. The susceptibility of *A. maculatus* to the three cynomolgis is illustrative of the type of difference noted. In this mosquito, the Cambodian strain developed both oocyst and sporozoite infection in nearly all those fed. *P. c. bastianellii* developed to the oocyst stage in nearly all but only 60-80% showed sporozoite infection and the Gombak strain was similar to *P. c. bastianellii* except that less than 10% of the mosquitoes developed a sporozoite infection. Combination of these data with the rate of sporogony gave an accurate identification of the strain in about one-third of the time required by studying the schizogonic cycle. Numerous other differences in the susceptibility of various Malayan anophelines were demonstrated in chart form.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. V. ZAMAN and MR. N. VISUVALINGAM
(Department of Parasitology, University of Singapore)

Acridine Orange Staining of Some Blood Parasites

The staining of tissues with Acridine Orange is widely used for exfoliative Cytology in Gynaecology and the malignancies of the respiratory tract (Bertalanffy and Bertalanffy, 1960). The stain gives a bright polychrome picture when examined under a fluorescent microscope. The method depends on the differentiation of RNA and DNA by Acridine Orange. The DNA of the nucleus gives a green or yellow fluorescence, while the RNA of the cytoplasm gives an orange to bright red fluorescence. The proliferating malignant cells are readily characterized because of the high RNA content.

In case of blood parasites the stain has been used by Rothstein (1958), who found it a useful screeming procedure for detecting various parasites.. In this study we have tried the stain on Microfilaria, Plasmodia and Toxoplasma. In all cases the stain was used after fixing the cells and the procedure given by Humason (1962) was followed.

The microfilaria of *Brugia pahangi* fluoreced brightly, were easily recognizable and their internal structures could be clearly differentiated. The cytoplasm gave a bright orange fluorescence indicating a high RNA content. The nuclei gave a bright yellow fluorescence indicating a high DNA content.

In case of *Plasmodium berghei* the parasites were visible as localized fluorescent areas in the infected cells and were readily recognizable. The internal structure was, however, not clear enough to identify the different stages.

In case of *Toxoplasma gondii* the parasites showed a beautiful polychrome staining. The cytoplasm was stained orange to bright red and the nucleus bright yellow.

REFERENCES

Bertalanffy, L. von and Bertalanffy, E. D. (1960). A new method for cytological diagnosis of Pulmonary Cancer. **Ann. N.Y. Acad. Sci. 84,** 225-238.

Humason, G. L. (1962). **Animal Tissue Techniques.** W. H. Freeman & Co., San Francisco.

Rothstein, N. (1958). Vital staining of blood parasites with Acridine Orange. **J. Parasitol. 44,** 588-596.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. F. L. DUNN
(University of California International Center, for Medical Research, IMR, Kuala Lumpur)

A Malayan bat trypanosome resembling **T.cruzi** and **T.vespertilionis**

Trypanosomes have been known as parasites of bats since at least 1899 when Dionisi described such a parasite, without naming it, from a species of *Miniopterus* in Italy. In 1904 Battaglia described a small trypanosome from a European *Vesperugo* as *Trypanosom vespertilionis*. This name has survived and has been applied to many trypanosomes of African, American, and European bats. Other *T.vespertilionis*-like forms have been described under a variety of names: it is doubtful whether many of these specific names are valid. (WENYON — PROTOZOOLOGY I, pp. 479-482. 1926).

T.vespertilionis is of outstanding interest because of its close relationship to *T.cruzi*, the parasite responsible for Chagas disease in the Americas. The parasites are similar morphologically and in having a tissue developmental cycle which sets them apart from other mammalian trypanosomes. South American workers have concluded that *T.cruzi* and *T.vespertilionis* should be placed in a separate genus, *Schizotrypanum*, because of the unique life cycle and morphological characteristics.

Although *T.vespertilionis*-like parasites are now known from Europe, Africa, the Americas, and Australia (*T.hipposideri* Mackerras, 1959) none have been reported to date from any part of Asia. Nor has a *T.cruzi*-like trypanosome been found in any other Asian mammal — in Asia. (There are a few *T.cruzi* records for primates imported from Asia to Europe and North America, but in all cases the infections could have been acquired in transit or after arrival in the zoo or laboratory.)

Most of the other bat trypanosomes constitute a group of related species, resembling *T.megadermae*, found to date only in Africa and Latin America. The only trypanosome reported for an Asian bat was found by Donovan (about 1904) in an Indian "flying fox" (*Pteropus medius*). Probably this parasite is related to or conspecific with *T.pteropi* Breinl, 1913 of Australian flying foxes. This trypanosome is in some morphological respects intermediate between those of the *T.megadermae* and *T.vespertilionis* groups. It is large and the nucleus lies near mid-body (as in *T.megadermae*), but there is a large subterminal kinatoplast (as in *T.vespertilionis*).

The subject of this demonstration is a small trypanosome found in two of 31 *Tadarida johorensis* (free-tailed bats) collected in October 1963 at Ampang, near Kuala Lumpur, Selangor. A search of the available thin and thick blood films disclosed about 15 intact trypanosomes of which 10 were suitable for measurement. Studies have not so far included examination of tissues from these bats for possible developmental stages. Like *T.pteropi*, the trypanosome of *Tadarida* is in certain respects intermediate in morphology between the two major bat groups. It resembles *T.vespertilionis* in having a large subterminal kinetoplast (equally large in thick and thin films), but it is somewhat larger than typical members of the group. The most striking difference is in the position of the nucleus, which is posterior to mid-body in all specimens. Measurements (ranges, in microns) are as follows: total length — 20.5 - 23.5, body length — 13 - 16.5, flagellum — 5 - 7.5, posterior end to mid-nucleus — 5 - 6, mid-nucleus to anterior end — 7.5 - 11.5, maximum breadth — 1.5 - 2, diameter of kinetoplast — 0.75, length of nucleus — 1.5 - 2. The parasite probably must be described as a new species, particularly because of the position of the nucleus, but in some morphological characters it is closer to *T.cruzi* than to the other known trypanosomes of bats.

(This brief study has been supported in part by U.S. Public Health Service ICMRT Grant GM-11329-03; in part by the Office of the Surgeon General Dept. of the Army.)

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

PROF. R. S. DESOWITZ
(Department of Parasitology, University of Singapore)

and

DR. J. J. SAAVE
(Malaria Service, Department of Public Health, Territories of Papua and New Guinea)

A Study on the Natural History of Immunity to Malaria in Protected and Unprotected Populations of Australian New Guinea

A tanned sheep erythrocyte hemagglutination test for the measurement of antibody in malaria has been described by Desowitz and Stein (*Trans. R. Soc. trop. Med. Hyg. 1961* 56,) and Stein and Desowitz (WHO/Mal/393, 1963; *Bull. W.H.O.* in press). It was of obvious interest to apply the test to a population living in an area where malaria is endemic in order to correlate serological results with our knowledge of immunity gained from clinical and epidemiological studies. The area chosen was the Maprik District of Australian New Guinea. A considerable amount of epidemiological data was already available on the population (Peters, W. 1960. *Trans. R. Soc. trop. Med. Hyg., 54,* 242) and some village groups have been subject to malaria control. There is also a programme to bring the population living under conditions of holoendemic malaria under protection by spraying and mass drug distribution. It is possible therefore to obtain some idea of the changes of the immunological picture of a population after control measures have been instituted.

The population of a group of closely associated villages (Unprotected Group) were presurveyed in June 1963. Parasitaemia rate, spleen rate, and liver enlargement were assessed. In August the same group was again surveyed and at the same time sera was obtained for serological studies. An *ad hoc* survey was also made of a group (Protected Group) in which malaria has been controlled since 1959 by twice yearly DDT spraying and mass distribution of a chloroquine and pyremethamine mixture. The hemagglutination test was carried out in Singapore. Two antigens were used, *Plasmodium cynomolgi* and *P. coatneyi*, since previous studies indicated the former to be antigenically related to *P. vivax* and the latter to *P. falciparum*. The results are shown in the two figures demonstrated. It will be seen that in the Unprotected Group there is a gradual rise in average hemagglutination titre from infancy to adulthood. Comparison of the 1-2 yr group with the adults shows that this increment is approximately 5 times with *P. cynomolgi* antigen and 3 times with *P. coatneyi* antigen. The differences in average titre between the Unprotected Group and Protected Group are striking. For the 1-2 yr age group the average titre of the Protected population approximately is 1/10 — 1/15 that of the Unprotected population, for the 3-4 yr group 1/7 — 1/20, and for adults about 1/2. Unfortunately the number of sera collected from the Protected Group was limited and not all age groups included.

In September 1963 the Unprotected Group was given mass drug treatment and the villages sprayed with DDT. It is planned to make similar yearly surveys of this population to determine the effect of malaria control on the immunological picture.

Age group	Number tested	Average titre (reciprocal) *P. cynomolgi* antigen	Average titre (reciprocal) *P. coatneyi* antigen
UNPROTECTED GROUP			
1 - 2 yrs	28	2,271	3,078
3 - 4 yrs	48	7,541	5,470
5 - 6 yrs	50	5,416	5,742
7 - 9 yrs	44	10,386	7,004
10 - 15 yrs	54	8,120	7,348
Adults > 16 yrs	232	11,190	8,181
PROTECTED GROUP			
1 - 2 yrs	26	146	338
3 - 4 yrs	18	300	711
Adults	27	4,251	5,911

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

PROF. R. S. DESOWITZ
(Department of Parasitology, University of Singapore)

and

DR. J. J. SAAVE
(Malaria Service, Department of Public Health, Territories of Papua and New Guinea)

A comparison of the serum proteins of a population living under conditions of holoendemic malaria and one subject to malaria control

The immunological survey and the nature of the Protected and Unprotected Groups were described in our demonstration I. Serum proteins were analyzed by the Antweiler micro-Tiselius method of moving boundary electrophoresis. A graph comparing average gamma-globulin levels in both groups and a table showing average values for all serum protein fractions was demonstrated. In the unprotected group the gamma-globulin was relatively high for all ages, ranging from 2.03 to 2.52 grams %. There is apparently little difference between age groups although the lowest value 2.03 grams % was found in the 1-2 yr olds and the highest 2.52 grams % in the adults. In the three age groups of the protected population, 1-2 yrs, 3-4 yrs, and adults, that were tested the average gamma-globulin level was considerably lower, being 1.33, 1.40 and 1.80 for the three groups respectively. There does not appear to be any significant differences in the beta and alpha globulins or the albumin between the unprotected and protected groups except that albumin level in the adults of the former group might be slightly decreased.

Age group (yrs)	No	Average serum proteins (grams %)				
		gamma-globulin	beta-globulin	alpha-globulin	Albumin	Total protein
1 - 2 Malarious group	16	2.03	1.09	0.93	3.35	7.37
1 - 2 Protected group	8	1.33	1.04	1.18	3.90	7.45
3 - 4 Malarious group	30	2.37	0.94	0.90	3.25	7.44
3 - 4 Protected group	14	1.40	0.86	1.16	3.48	6.90
5 - 6 Malarious group	26	2.44	1.02	0.75	3.21	7.42
7 - 9 Malarious group	24	2.25	1.11	0.85	3.12	7.33
10 - 15 Malarious group	29	2.22	1.14	0.75	3.25	7.37
Adults Malarious group	47	2.52	1.37	0.58	2.98	7.58
Adults Protected group	23	1.84	1.18	0.85	3.46	7.36

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

MRS. B. STEIN and Prof. R. S. DESOWITZ
(Department of Parasitology, University of Singapore)

Haemagglutinating Antibodies in Simian Malaria

Sera of 28 rhesus monkeys were obtained from Dr. McWilson Warren and the late Dr. Don Eyles of IMR, Kuala Lumpur.

These monkeys were infected with 8 different simian malarias.

These sera were posted by air mail in ice-cooled thermos flasks without preservatives and tested shortly after arrival.

The method of haemagglutination test employed was essentially the same as described by us in 1962 and 1963 except that the absorption of the sera was carried out with formalized tanned sheep cells, instead of with fresh sheep cells.

Figures given in the following table are average titres for monkeys infected with same species of malaria.

In case of *P. gonderi* we had serum of one monkey only, in the rest there were sera of 2-5 monkeys.

Sera from two monkeys known not to have any malaria infection are included as normal controls.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

MR. R. B. GRIFFITHS
(United Nation's Food and Agricultural Organization)

Leucocytozoon caulleryi in the domestic fowl in Southeast Asia

Within recent years several serious outbreaks of acute leucocytozoonosis in fowls have been reported from countries in southeast Asia and from Japan.

The author's experience has been in Burma where the species involved in outbreaks in 1962 and 1963 has been *Leucocytozoon caulleryi*.

The demonstration comprised a description of the disease caused by *L. caulleryi* infection.

The disease, which generally affects birds at 3-5 months of age, is acute. Before death, there may be expectoration of blood-stained ropy mucus from the respiratory tract, or the passage of blood through the cloaca, but very frequently birds die without showing symptoms. The mortality rate is frequently about 20% in affected flocks, but this figure may be exceeded.

At autopsy, haemorrhages may be observed in almost all of the tissues but the most serious and most extensive haemorrhages are found in the lungs and kidneys. There may be gross haemorrhage from the kidneys into the peritoneal cavity. Megaloschizonts are regularly present in the organs and tissues which show haemorrhages. The megaloschizonts are found therefore throughout the lungs and kidneys as well as in the liver, spleen, pancreas, ovaries, testes, on the mucous membranes of the alimentary and respiratory tracts, on all the serous membranes, the meninges, and in the subcutaneous fascia.

In the visceral organs, the megaloschizonts frequently occur as scattered individual bodies measuring up to 300 microns in diameter; each is therefore just visible to the naked eye as a cream coloured round structure. They also occur in groups in these organs, but the tendency to the formation of collections of megaloschizonts is more pronounced on the mucous and serous membranes. A group of megaloschizonts, which may consist of six or more of these structures, is frequently oval in shape and may reach 1.5 mm x 2 mm or more in size. A group of megaloschizonts frequently has a variegated cream and red appearance which results from the entry of blood into the interior of megaloschizonts that have discharged their merozoites.

To demonstrate megaloschizonts in the tissues it is not necessary in routine laboratory diagnosis to resort to histo-pathological methods. Crush preparations, prepared by crushing small portions of lung, kidney or other tissues between two glass slides, serve adequately for the demonstration of megaloschizonts by naked eye examinations or observation under low power magnification.

Blood smears stained with a Romanowsky stain show one or more of the following, depending upon the stage of infection when examination is made:

(a) Merozoites in the plasma.

(b) Merozoites in the erythrocytes. Frequently there is multiple invasion by the merozoites which each measure about 1.5 μ in diameter.

(c) Developing gametocytes in immature erythrocytes. At this stage the parasite causes a round distortion on the host cell in contrast to spindle-shaped distortion which is seen with certain other species of *Leucocytozoon*.

(d) Extra-cellular mature gametocytes. At this stage there is usually no trace of host cell, although occasionally a remnant of the host cell cytoplasm, but not the nucleus, may be found. The mature gametocytes are round

or sub-spherical and measure approximately 12-14 μ in diameter.

The demonstration included an exhibit of megaloschizonts and haemorrhages in the kidney, a group of megaloschizonts under the serosa of the small intestine, and blood smears showing the stages described above.

The assistance of U Tha Khin, Parasitologist, Veterinary, Educational and Research Institute, Burma, who has collaborated with the author in the investigation of leucocytozoonosis is acknowledged.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

PROF. A. A. SANDOSHAM and MR. S. SIVANANDAM
(Division of Malaria and Filariasis, IMR, Kuala Lumpur)

Filarial worms in the mousedeer **Tragulus javanicus**

Two filarial infections occur in the local mousedeer (*Tragulus javanicus*, sometimes in the same animal as in this case.

Setaria javensis was first described in 1922 by Vevers from a female worm obtained at the London Zoo. Sandosham (1953) described the male from a mousedeer that died in Kuala Lumpur. Since then several specimens have been collected from mousedeer in Malaya both from the East Coast (Pahang) and Bukit Mandul (Selangor).

From some of the animals examined specimens of *Papillosetaria* sp. were also obtained. Two species belonging to this genus have been described both from Java, *P. traguli* Vevers, 1922 and *P. veversi* Maplestone, 1931.

Demonstrations showed adult *Setaria javensis* and adult *Papillosetaria* sp. A thick blood film showed the marked differences in the microfilariae of the two filarial worms.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

PROF. A. A. SANDOSHAM and MR. S. SIVANANDAM
(Division of Malaria and Filariasis, IMR, Kuala Lumpur)

A new filarial worm from the flying lemur (Cynocephalus variegatus)

These filarial worms were obtained from the subsutaneous tissues in the back from flying lemurs collected by Dr. F. L. Dunn in N. Borneo and Kepong (Malaya). One flying lemur obtained in Perlis (N. Malaya) showed microfilariae in the blood.

The adult females measure from 85 to 155 mm. in length while the male is only 20 mm. long. The microfilariae measure from 120 - 125 μ in length and 2.5 - 3.5 μ broad.

The specimens are being studied in the Division of Malaria and Filariasis Research.

The demonstration showed adult worms and microfilariae.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

W. H. CHEONG and A. H. Omar
(Division of Entomology, IMR, Kuala Lumpur)

Preliminary figures on the developmen of **B. malayi** in a laboratory and a natural vactor

Laboratory bred mosquitoes, *Aedes (s) aegypti* were fed on a *B. malayi* carrier (cat) with a blood concentration of 16.1 microfilariae per cmm, and were dissected at daily intervals to observe the rate of growth. A proportion of the developing larvae are measured and the figures compared with those obtained by Wharton (1957) for the natural vector *Mansonia dives*. The number of larvae present and the stage of development will also be recorded eventually. The growth and appearance of the larvae correspond fairly well with those of Brug (1931) Feng (1936) and Wharton (1957) with a minor difference in the time of development.

However, it should be noted that this is the first of a series of feedings to be carried out and therefore the findings are not as yet final. Wharton measured stage I larvae in saline and stages II and III in Bless fluid. The present study differs in that all the stages were dissected and carefully teased out in serum saline and fixed in iodine fumes, allowed to dry and stained in giemsa before measurement.

It was noted that development in *Aedes (s)* aegypti followed a similar pattern to that of *M. dives* but from about 6.5 days onwards development seemed to be slower than that in *M. dives* so that there were many which were still in their Stage II at 9.5 and 10.5 days.

Furthermore quite a number of chitinised Stage I and II worms were noted. These obviously were all dead. Confirmation of this was made on the 12th and 13th day by dissection when not many infective larvae were found, which indicated that although many microfilariae may be ingested initially, not many developed through to stage III.

In may seem therefore, that the laboratory vector is not as good a host as the natural vector from this experiment. However, further investigation has to be made before we can be sure. The one great advantage *Aedes (s) aegypti* has over *Mansonia* as a laboratory vector is that it only takes a week to breed large numbers where as *Mansonia dives* takes about a month with special care.

REFERENCES:

Wharton, R. H. (1057). Observations on the development of **Wuchereria malavi** in **Mansonia (Mansonioides) longipalpis. Ann. trop. Med. Parasit., 51,** 278.

Brug, S. L. (1931). Filariasis in the Dutch East Indies. **Proc. R. Soc. Med., 24,** 663.

Feng, L.C. (1936). The development of **Microfilaria malayi** in **A. hyrcanus** var. **sinensis Wied. Chinese Med. J.,** suppl. I, 345.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. PAUL F. BASCH
(University of California International Center for Medical Research, IMR, Kuala Lumpur)

Some Malayan Schistosomes

The schistosome trematodes are of great interest because of the three important species which parasitize man in various parts of the world. Here in Malaya human schistosomiasis has never been reported, but there are several species of avian and mammalian schistosomes. The cercariae of these non-human parasites can cause a dermatitis in man.

The life cycle of a species of *Trichobilharzia* from padi fields in Negri Sembilan has been completely worked out, and drawings of most of the stages are on display. The species has not yet been identified with certainty, but it is hoped that this will be accomplished soon. The worms develop and mature very rapidly, producing viable eggs in ducks in some cases only 13 days after penetration of cercariae. The ducks will lose their infection (i.e., stop shedding eggs) in some weeks. Immunity to re-infection has not yet been investigated. In mice this avian parasite can cause large haemorrhages and considerable damage to the lungs, but it will not develop to the adult stage. This demonstrates at least that the parasite can penetrate further than the skin in some mammals, and leaves open the question of possible systemic involvement in man.

Another parasite, of the genus *Schistosoma,* is now under study. *Indoplanorbis* snails shedding large numbers of cercariae have been collected within five miles of the I.M.R. laboratory, and mamals of several species have been exposed to the larvae. (What appears to be the same trematode has also been found in Negri Sembilan Indoplanorbis snails). Infection of mice and hamsters in easy. Hundreds of adult worms may be removed from the portal vein, mesenteric vein, and liver some time after a single exposure to cercariae. The worms do not appear to become sexually mature in these hosts, however. A *Macaca mulatta* monkey failed to become infected after repeated exposures over a period of about two months. It has been possible to hatch living, active miracidia from eggs in cow dung collected near the infected snails, but the eggs themselves have not yet been observed microscopically. Thus the species of the worm cannot yet be stated with complete confidence, but it appears to be *Schistosoma spindale* (or *spindalis*) Montgomery, 1906.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. E. BALASINGAM
(Department of Zoology, University of Malaya, Kuala Lumpur)

The Parasitic growth of **Placocorus lotoris** (Schwartz, 1925) Webster, 1956 (Ancylostomatidae: Nematoda)*

The parasitic growth of strongylid namatodes have been well studied in the past. Most noteworthy are the investigations on *Heligmosomum muris* by Yokogawa (1922) *Nippostrongylus muris* by Twohy (1956) and *Cooperia curticei* by *Sommerville* (1960). The present study concerns the parasitic growth of *Placocorus lotoris* which is an intestinal nematode parasitic in North American raccoons and skunks.

Infective larvae obtained from faeces-charcoal cultures by Baermann technique was administered orally to raccoons. The animals were killed at specific intervals after infection and examined for presence of parasitic stages. Mean measurements of the parasitic larvae are shown in Table I. Figure 1 represents the growth curve drawn from these measurements.

(Fig. 1, not reproduced here).

Measurements (mm.)

Age	Parasitic third stage (sex not differentiated)	Fourth Stage		Fifth Stage	
		Male	Female	Male	Female
1 day	0.628	–	–	–	–
2 days	0.621	–	–	–	–
3 days	0.639	0.724	0.885	–	–
5 days	0.647	1.537	1.812	–	–
7 days	–	2.043	2.216	–	–
10 days	–	2.012	2.256	2.423	2.710
15 days	–	–	–	3.496	3.972
20 days	–	2.096	2.302	4.025	4.753
25 days	–	2.147	2.322	5.272	6.252
30 days	–	–	–	5.652	6.802
35 days	–	–	–	5.781	6.912
40 days	–	2.152	2.286	5.602	7.081
3 months	–	–	–	5.706	7.128

Table I — Growth of parasitic larvae

It is observed that the parasitic third stage larvae do not undergo any increase in size. They enter the fourth stage in 48-72 hours after infection. The fourth ecdysis and the associated lethargus occur between 7 and 10 days. Although most of the larvae undergo normal development to maturity in about 30 days, some of the fourth stage larvae appear to be unable to complete development and to attain the fifth stage. This phenomenon has been observed among parasitic stages of *Haemonchus placei, Ostertagia circumcinta* and *Cooperia curticei* (Bremner 1956, Sommerville 1954, 1960). Sommerville (1960) pointed out the

uncertainity as to whether retarded development was the consequence of an initially unsuitable environment or an environment rendered unfavourable as a result of infection. Whichever the case may be, the fact that the majority of the larvae undergo normal development of maturity while others are retarded indicates that among a given lot of larvae, there is a considerable range in variation in their ability to adjust themselves to the host environment and finally to reach the adult stage.

REFERENCES

BREMNER, K.G. (1956) **Austral. J. Zool.** 4, 146-151.
SOMMERVILLE, R.I. (1954) **Austral J. agric. Research,** 5, 130-140.
SOMMERVILLE, R.I. (1960) **Parasitology** 50, 261-267.
TWOHY, D.W. (1956) **Amer. J. Hyg. 63,** 165-185.
YOKOGAWA, S. (1922) **Parasitology, 14,** 127-166.

* Studies undertaken at the Institute of Parasitology, McGill University, Montreal in 1962/63.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

PROF. LIE KIAN JOE and MISS T. UMATHEVY
(Division of Virus Research & Medical Zoology IMR, Kuala Lumpur)

An undescribed 43-spined **Echinoparyphium** species and its life-cycle

The life-cycle of an undescribed 43-spined *Echinoparyphium* species is established in the laboratory. The first intermediate host is the fresh water snail *Lymnaea rubiginosa*. The sporocysts develop in the heart cavity. The first cercariae are released 22 day after exposure of the snail to miracidia. The metacercariae develop in the same snail and in other fresh water snails. The adults live in the small intestine of pigeons, ducklings and birds such as the spotted munia (*Lonchura punctulata fretensis*), the black-headed munia (*Lonchura atricapilla sinensis*) and the Java sparrow (*Padda oryzivora*). The adult worm, rediae, cercaria, metacercaria and egg were shown. Drawings of the adult worm and of all larval stages were also exhibited. The complete life-cycle and morphology of the worm and the larval stages will be published later.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

PROF. LIE KIAN JOE and MISS T. UMATHEVY
(Division of Virus Research & Medical Zoology IMR, Kuala Lumpur)

An undescribed 37-spined **Echinostoma** species and its life-cycle

The complete life-cycle of an undescribed 37-spined *Echinostoma* has been worked out under experimental conditions. The adult worm as well as egg, miracidium, rediae, cercaria and metacercaia were shown. Drawings of the mirecidia, sporocyst, rediae, cercaria, metacercaria and adult worm were also exhibited. The first intermediate host is the fresh water snail *Lymnaea rubiginosa*. The sporocyst develops in the heart cavity. There are at least 3 redial generations. Cercariae are released 19 days after exposure of the snail to miracidia. They encyst in the same and in other fresh water snails such as: *Indoplanorbis exustus*, *Gyraulus convexiusculus*, *Bellamya ingallsiana* and *Pila scutata*. The adult worms develop in the rectum of pigeons, ducks, the little cuckoo-dove (*Macropygia ruficeps malayanum*), the black-headed munia (*Lonchura atricapilla sinensis*), the spotted munia (*Lonchura punctulata fretensis*) and the Java sparrow (*Padda oryzivora*). The complete life cycle as well as the morphology of the adult worm and the larval stages will be published later.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. K. RHODE
(Department of Zoology, University of Malaya, Kuala Lumpur)

Studies on the distribution of **Opisthorchis** in Malaya

In a previous survey of *Opisthorchis* in cats and dogs, conducted in 1960 and 1961, 3 out of 70 cats were found to be infected. None of the 13 dogs, then examined, was infected (Rhode, 1962, *Med. J. Malaya, 17*, 94). Since then, 99 other cats from various parts of Malaya have been examined. The combined results of these two surveys are given below.

Locality	Number of cats examined	Number of cats infected with *Opisthorchis*	Frequency of infection %
Kuala Lumpur	67	1	1
Kepong	48	8	17
Ipoh	30	3	10
Kota Bharu	15	0	0
Kampongs south of Kuantan on the Pahang River	9	0	0
Total	169	12	28

Table 1: *Opisthorchis* infections in Malayan cats.

The cats from Kuala Lumpur were from Petaling Jaya, Kampong Bahru, Sentul and the centre of town. Of these, only one cat (from Sentul) harboured 1 *Opisthorchis*. The intensity of infection in cats from Ipoh was 1, 7 and 8 (average 5), that in cats from Kepong 1, 2, 2, 2, 2, 10, 14 and 16 (average 6). Most cats were also infected with another liverfluke, *Platynosomum fastosum* Kossack, 1910.

The low intensity of the infections reinforce the author's opinion (Rhode, 1962, Med. J. Malaya 17 : 94) that *Opisthorchis* is of less importance in Malaya than in Thailand, where the parasite is widely distributed among cats, dogs and man, especially in the North-Eastern Provinces (Sadun, 1955, *Amer. J. Hygiene*, 62, 81-115). Nevertheless, because of the relatively high frequency of infection in cats from Kepong and Ipoh, it seems to be advisable to look for *Opisthorchis* infections in future surveys among the human population. In this connection it should be mentioned that Sadun found only 4 out of 20 cats and 6 out of 94 dogs infected in areas of high endemicity in the northeast of Thailand, where approximately one fourth of the human population is infected.

There is considerable confusion about the taxonomic status of the South-East Asian form of *Opisthorchis* from cats, dogs, and man. According to Sadun, the form occurring in Thailand must be included in the species *O. viverrini* (Poirier, 1886) Stiles and Hassall, 1896, because the ratio of the mean length over the mean breadth of the eggs is 1.75 (in *O. viverrini* 2.0, in *O. felineus* 2.75). Furthermore, as in *O. viverrini*, the testes are located in close proximity to the caudal end of the worm, and the mean length of the worms and the eggs is similar in the specimens from Thai-

land and *O. viverrini*. Bisseru (1957, *J. Helminth, 31*, 187-202) considers *O. viverrini* as synonymous with *O. felineus* (Riv., 1884) Blanch, 1895, while Gupta and Pande (1963, J. Helminthology 37 : 291-298) consider *O. tenuicollis* (Rud., 1819) Stiles and Hassall, 1896 as the valid species, and *O. felineus* and *O. viverrini* to be synonymous. It is perhaps best to leave open the specific status of the Malayan form (or forms) until such time as the life-cycle and the developmental morphology of the parasite are known and can be compared with those of forms from elsewhere.

5 experimental groups, each consisting of 5-10 individuals of the snail *Digoniostoma pulchellum* (Bens.), were exposed to miracidia of *Opisthorchis* from Malayan cats. This snail was chosen, because it is closely related to *Bithynia leachi*, the intermediate host of the European and North-Asiatic *Opisthorchis*. Cercariae of *Opisthorchis* did not emerge from any of these snails, and, on dissection, no larval *Opisthorchis* were found. In addition, approximately 400 specimens of *Digoniostoma pulchellum* from Kepong were examined for natural infections of *Opisthorchis*. None of these was infected.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

MR. WONG SOON KAI
(Lau Keng Howe Hospital, Sibu, Sarawak)

and

PROF. LIE KIAN JOE
(Division of Virus Research & Medical Zoology IMR, Kuala Lumpur)

Poikilorchis eggs obtained from a second case of subcutaneous retro-auricular abscess in Sarawak

Demonstration of eggs obtained from a retro-auricular abscess occurring in a Dyak boy, aged 10. This is the second case of retro-auricular abscess caused probably by a trematode of the genus *Poikilorchis*. The first case occurred in a 8-year old Dyak boy. (Lie Kian Joe, et al. 1962, *Med. J. Malaya*, 17, 37-39).

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

MR. C. K. OW YANG, MR. B. L. LIM and PROF. LIE KIAN JOE
(Division of Virus Research & Medical Zoology, IMR, Kuala Lumpur)

Some Observations on the presence of Immunity in Rats to **Angiostrongylus cantonensis** (Chen)

Evidence from rats in the laboratory indicates that these animals appear to develop immunity after initial exposures to small numbers of *A. cantonensis* larvae. A dosage of 500 larvae fed to each of 5 white rats that have received 7 monthly exposures of 10 larvae each prior to testing, produced an average recovery of 20 worms per host, with no apparent signs of ill health in any of the rats at the time of necropsy two months after feeding. This significantly differs from the control where an average of 190 worms per host was obtained, with all the rats dead within 23-31 days after exposure.

Evidence of immunity also appears to occur in rats in nature. An average of 8 worms per rat was recovered from 57 infected *R. jalorensis* from an oil-palm plantation. When 10 infected *R. jalorensis* from the same locality were given an inoculum of 300 *A. cantonesis* larvae each, the average recovery was 16 worms per rat. The same inoculum given to laboratory-bred *R. rattus jarak* (closely related to *R. jalorensis*) and 10 white rats produced average recoveries of 94 worms per rat in both cases.

Since in nature, the number of worms recovered from any single *R. jalorensis* has never even approached that expected in the laboratory, it may be surmised that these rats had some degree of immunity, inherited or acquired or both. These possibilities are being investigated.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. M. A. FERNANDO and DR. H. A. WONG
(Departments of Parasitology and Biochemistry University of Singapore)

Demonstration of the route of absorption of glucose by **Ancylostoma caninum** using autoradiographic techniques

During the course of investigations into the glucose metabolism of *Ancylostoma caninum* it became necessary to determine the route of absorption of glucose in this nematode — through the cuticle or via the gut membrane.

Autoradiographic technique was used to show the uptake of radioactive glucose by the parasite. This method allows the detection of atoms of an element in minute quantities and its location in the tissues can be studied.

A. caninum was incubated in dog serum containing radioactive glucose (4 μc per 1.5 mg glucose) for $\frac{1}{2}$ hour at 37°C and washed several times in saline to remove extraneous glucose. The parasites were cut into lengths of 2-3 mm and fixed in Carnoy's fluid at 4°C. Paraffin sections of 8 μ were prepared in the usual manner and stripping film method of autoradiography was employed. The slide with the film emulsion was developed after 14 days and the sections stained in Erlich's haematoxylin.

The photomicrographs of the autoradiographs show clearly that *A. caninum* absorbs C-14 glucose mainly if not entirely via the gut membrane with hardly any absorption through the cuticle.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. McWILSON WARREN and DR. GORDON F. BENNETT
(U.S.P.H.S., Far East Research Project, IMR, Kuala Lumpur)

and

MR. W. H. CHEONG
(Division of Entomology, IMR, Kuala Lumpur)

Natural Plasmodial Infections in **Mansonia (Coquillettidia) crassipes**

Field investigations to determine the vectors of simian malaria have been conducted in various ecological areas of Malaya. Such investigations involved trapping with animal baited net traps both on the ground and in the forest canopy; daytime resting catches; and bare leg catches. All mosquitoes were returned to laboratories at the Institute for Medical Research and examined for malaria parasites. Such examinations were carried out with culicines as well as anopheles.

In October, 1963 field studies were conducted at Pacific Tin north of Kuala Lumpur. This is an area of extensive fresh water swamp forests where a large percentage of monkeys are infected with malaria. Large numbers of the *Anopheles umbrosus* group as well as members of the genus *Mansonia* were found. During the process of routine dissections one *Mansonia crassipes* was found to be infected with a malaria parasite. Both the gut and the salivary glands were positive. Only 39 mosquitoes of this species had been dissected from this area and all of the remainder have been negative for malaria.

The sporozoites from the positive mosquito were wet-fixed in iodine fumes and stained with Giemsa. On examination the sporozoites were found to be similar both in size and shape to those of *Plasmodium traguli*. The oocysts on the midgut were also similar to those of *P. traguli*. It is interesting that *A. umbrosus* mosquitoes from the Pacific Tin area are frequently infected with this parasite. The mosquito stages of *P. traguli* are quite different from those of primate or bird malarias which have been studied in this laboratory. To our knowledge, there are no reports of non-anopheline vectors of mammalian malaria parasites.

It is interesting to note that this mosquito was also infected with *Setaria* sp. larvae. This is a very common helminth infection in the Malayan mouse deer.

Giemsa-stained preparations of sporozoites *P. traguli*, *Plasmodium* sp. (from Malayan chickens) and the tentatively identified parasite from *Mansonia crassipes* were demonstrated.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. DOUGLAS E. MOORHOUSE
(World Health Organization, Kuala Lumpur)

Enchanced damage to attap roofs by the caterpillars of **Herculia nigrivitta,** following house-spraying with DDT

In March 1961 house-spraying with a DDT emulsion was started in the area of the malaria eradication pilot project in a part of the State of Selangor. It was designed to give a deposit of two grammes technical DDT on all sprayable surfaces. These were defined as the internal walls and roofs of the houses, (to a height of at least ten feet), the backs and undersides of all pieces of furniture; and where the house was raised on stilts, the underfloor area. Outside porches and the eaves were also sprayed, as were adjoining animal shelters. Since the start spraying has been repeated at six-monthly intervals. Five cycles have now been completed (December 1963). After the second cycle of spraying was finished, (June 1962) complaints were received that one result of the spraying was that the attap roofs of many houses were rapidly being destroyed by caterpillars. Field investigations confirmed that this was the case.

Attap the common thatch of rural Malayan houses, is made from the fronds of either the sago palm (*Metroxylon sagus*), or the fronds of *Nippah fruticans*, a brackish-water palm specially cultivated for thatching purposes. The normal life of such a thatched roof is from three to seven years, depending upon the way in which the attap is laid, whether it has been soaked in running water before being used, and whether it is made from the nipah or the sago palm; sago lasted the longest but is more expensive. Reliable evidence was produced that after DDT spraying roofs less than one year old were in holes and were ruined. This has been confirmed by subsequent observations.

The caterpillar responsible for the damage was identified as that of *Herculia nigrivita*, Walker, a small blackish-brown moth. This caterpillar is a well-known pest of attap in Malaya, but usually it is present in small numbers and the damage caused is small when compared with the normal rotting processess which take place in the attap. Most of the house-holders readily admitted that the caterpillar was present in their roofs before the start of spraying. Damage after spraying was found to be most severe in outside porches, the eaves, and other unenclosed places. It was moderately severe in smoke-free living rooms, and was minimal in kitchens where open fires were used.

Caterpillars taken from the attaps were found to be resistant to DDT. Forty were exposed to 4% DDT susceptibility test papers for one hour, and were then placed in gauze-covered jars and fed on DDT-sprayed attap for ten days. There was no mortality. They were found to be moderately susceptible to both BHC and dieldrin. Cheng (1963) has recently reported this same problem of increased damage after spraying from North Borneo, but he has evidence that DDT avoidance plays a part in Borneo. There is no such evidence from Malaya, and the caterpillar will readily feed on DDT-sprayed attap.

Examination of the remains of pupal cases of *Herculia nigrivitta* from old attap shows that many of them were parasitised. There is every reason to suppose that in Malaya, as in North Borneo (Cheng, 1963), this pest is naturally kept under a biological control by a hymenopterous parasite (*Antrocephalus* sp.), but that the controlling mechanism is susceptible to DDT whilst the caterpillar is resistant. Examination of both caterpillars and pupae from the sprayed area after the start of spraying has not shown the presence of the parasite.

Damage is not uniformly distributed over the whole sprayed area, but is concentrated into

certain areas. The reasons for which are not known. For survey purposes the area of the pilot project was divided into rectangles each of $8\frac{3}{4}$ square miles. During the fifth cycle of spraying operations 455 house-owners refused to allow the attap to be sprayed because of the damage; just over 60% of these refusals took place in four of the above-mentioned rectangles, probably about one-tenth of the inhabited area. Dieldrin was sprayed on the attap in one area where damage was intense (400 mgs/m^2) in an attempt to effect control. It reduced damage but did not provide a satisfactory answer to the problem.

It seems that this problem may interfere with any prolonged house-spraying campaign using DDT, and that it could well jeopardise any programme which demands a one hundred per cent total house coverage.

Demonstrations showed the caterpillars and adults of *Herculia nigrivitta* and specimens of damaged attap and photographs of huts affected from DDT-sprayed areas.

REFERENCE

Cheng, F. Y. (1963). Deterioration of thatch roofs by moth larvae after house spraying in the course of a malaria eradication programme in North Borneo. **Bull. Wld. Hlth. Ord., 28,** 136-7.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

MR. W. H. CHEONG and MR. A. GANAPATHIPILLAI
(Division of Entomology, IMR, Kuala Lumpur)

Preliminary observations on the aquatic stages of **Toxorhynchites** (Diptera : Culicidae) in Malaya

Mosquitoes of the genus *Toxorhynchites* have predatory larvae, and typically breed in tree holes or small containers. This makes them of great interest as potential agents of control in areas where they co-exist in such sites with larvae of mosquitoes or insects of medical importance.

As an example *T. splendens*, occurs very commonly in coastal areas of Malaya in Nipah palm bases and artificial containers. In Nipah palm bases at Rantau Panjang *Anopheles hackeri* also breed. The predacious larvae of *T. splendens* no doubt reduce the breeding of *A. hackeri* in this area. *A. hackeri* is an important vector of simian malaria in Malaya. Further evidence was obtained during our *Aedes aegypti* surveys when *Toxorhynchites* were taken from artificial containers in which *Aedes aegypti* and *A. albopictus* also breed.

In Malaya, ten known species of *Toxrhynchites* occur. The present study has been confined to the typical hill forest types from Ulu Gombak and Ulu Langat: *Toxorhynchites metallicus* and *T. magnificus*. They are regularly encountered breeding in living and split bamboos and in other natural containers rather like *T. brevipalpis* in Africa (Corbet, 1963).

The *Toxorhynchites* in Ulu Gombak live in association with two species of *Anopheles; A. asiaticus* in fallen split bamboos and *A. boniae* in living bamboos. Also found in this same association are at least six other genera of *Culicidae* namely *Tripteroides, Topomyia, Orthopodomyia, Aedes* (*Finlaya*), *Armigeras* (*Leicesteria*) and *Culex* (*Lophoceratomyia*). In the twenty larvae collected, all the four intars were included. These were placed in individual bowls and feeding habits were observed daily. The larvae of other mosquitoes were used in feeding them.

The main points of interest are as follows:-

1. The period from 1st instar to final emergence is between 21 and 24 days, of which the 1st instar takes two to three days, the 2nd and 3rd instar each taking three to four days, the fourth instar ten days, and the pupal stage to emergence between five and seven days.

2. It was found that during development, roughly 1, 2, 3 and 15 mosquitoe larvae were eaten per day by each of the 1st, 3rd and 4th instars.

3. Larvae were only killed for food by the 1st, 2nd and 3rd instars whereas the 4th instar not only ate numerous larvae during its ten day developmental period but also killed large numbers and left them uneaten. This was specially true during the last four days before pupation.

REFERENCE

Corbet, P. S. (1963). Observations on **Toxorhychites brevipalpis conradti** crunb. (Diptera: Culicidae) in Uganda. **Bull. ent. Res.** Vol. 54, Pt. I.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. GORDON F. BENNETT and DR. McWILSON WARREN
(U.S.P.H.S., Far East Research Project, IMR, Kuala Lumpur)

and

DR. DAVID W. ELLISON
(U.S. Army Medical Research Unit, IMR, Kuala Lumpur)

Chrysomia bezziana, a major hazard at the Malayan National Zoo

On December 17, 1963, a report was received from the Malayan National Zoo that one of the camels was suffering from an infected eye. On investigation, the orbit and lids were found to be greatly swollen and inflamed. Many second and third instars of a calliphorid were seen in and about the orbital tissue. Many muscids, calliphorids and sarcorphagids were seen feeding on the discharge from the orbit. A solution of approximately 7% chloroform-93% castor oil was applied to the area and the animal re-examined the following day. At this time, 10 dead larvae and nine living third instars were removed. The latter were allowed to pupate, pupation occurring on December 19 and 20. On December 27, five adult flies emerged, four adults emerging the following day. All adults proved to be *Chrysomia bezziana,* reported by Chandler and others as the most important myiasis-producing calliphorid of the South-East Asia region. The eye of the camel responded well to local treatment with an antibiotic powder, following the removal of the screwworms.

On December 28, a Ceylonese hog deer with a badly cut foot was examined. Several maggots were seen deep in the wound. During the examination and curetting of the wound on the second day, a large mass of calliphorid eggs were seen on the hoof near the wound. Ten to fifteen adult *C. bezziana* were attracted to the wound and blood from it during the examination. On January 4, 1964, the badly cut right forefoot of a wallabie was found to contain 14-16 second instar screwworms.

Routine inspection of the zoo premises revealed the presence of adults of *C. bezziana* in the kitchens, veterinary block, monkey cages, carnivore cages, many of the bird cages and the camel enclosures. The presence of this fly in such numbers about the grounds of the zoo emphasizes the fact that all cuts and abrasions suffered by the animals must be treated promptly to obviate further complications by myiasis. In addition, rigorous measures must be adopted to dispose of all rotting meat and offal that serve as attractants to the adult flies.

Adults and larvae of *C. bezziana* from the orbit of the camel were demonstrated.

REFERENCE

Chandler, A. C. **Introduction to Parasitology,** 9th New York: John Wiley and Sons. 1955.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

MR. W. T. CHELLAPPAH
(Dept. of Parasitology, University of Singapore)

Observations on Delayed Mating and Post-coital Impotence of Spermatozoa in **Aedes albopictus (Skuse) (Diptera : Culicidae)**

There is a lapse of time between mating and fertilizing of eggs in *Aedes albopictus*. Experiments were designed to determine the period of time that must first elapse before fertilization could take place.

Three cages were set up: Cage A females only; cage B males only; Cage C males and females together. All cages were maintained on honey and water unit the fifth day when cages A and C were offered a blood meal; unfed mosquitoes were removed. At 48 hrs (or more) after the blood meal, single females from cage A were exposed to approximately 50 males in cage B (these are referred to hereafter as "test females"). Mating took place almost immediately. The mated females were separated and maintained in 3" x 1" tubes and in each case matched with another female from cage C, set up in a similar tube as a control. The controls differed only in the time at which insemination occurred and probably to some extent in the number of inseminations received.

At various period after mating, so arranged that the period after blood meal was roughly the same in each case, oviposition was induced in the paired mosquitoes by de-alation under light anaesthesia. Batches of eggs were thus obtained which were laid at known periods after experimental mating, in each case matched with a batch from normally-mated control. When laying ceased, all females were dissected and the spemathecae examined for spermatozoa; where spermatozoa were absent or scanty, the test was discarded. At the same time, unlaid eggs were counted.

The eggs laid by each female were then transferred to individual slips of filter paper, counted and kept moist for 48 hrs. After this conditioning period, they were dried for 48 hrs and then placed in grass infusion, whereupon hatching usually took place almost immediately. At the end of 10-12 hrs, the hatched larvae were counted and a further check was made after 24 hrs in case of delayed hatching. Table I shows the mean number of eggs per female laid and retained, together with the total numbers developed and the proportions actually laid. These were grouped according to the periods which elapsed between mating and oviposition in the test mosquitoes. In all groups, the total number of eggs developed was less in the test mosquitoes than in the controls and in all but one group, the percentage of eggs actually laid was also lower. Statistical analysis shows both differences to be highly significant, $P < 0.01$.

Table I shows the hatching rates of eggs laid by single test mosquitoes, and in table II these are grouped according to the period between mating and oviposition and compared with the controls. It is clear that there was no fertilization of eggs which were laid less than seven hours after mating and very little before eight hours, after which there was a rapid increase in the proportion fertilized.

The apparent impotence of the spermatozoa during the period of the first eight hours after mating is clearly not due to any delay in their migration to the spermathecae. Spermatozoa were seen to be filling the spermathecae in as little as two minutes after mating. It would appear either that the spermatozoa require a period of maturation inside the spermathecae (perhaps a form of "acclimatisation") or that some hormone, essential to the act of fertilization is produced some hours after the stimulus of filing of the spermathecae.

TABLE I

Mean numbers of eggs/mosquito developed, laid and retained

Experimental Period*	No. of Mosquitoes	Test mosquitoes				Control			
		Eggs laid	Eggs retained	Eggs developed	% Eggs laid	Eggs laid	Eggs retained	Eggs developed	% Eggs laid
up to 6 hrs.	22	55	44	99	56	76	31	107	71
6 - 8 hrs.	8	51	35	86	59	84	23	107	79
8 - 10 hrs.	6	57	20	77	74	65	31	96	68
10 - 12 hrs.	6	55	31	86	64	85	21	106	80
Over 12 hrs.	10	53	40	93	57	86	18	104	83
TOTAL:	52	54	38	92	59	79	26	105	75

* Period elapsed between mating and oviposition

TABLE II

Hatching rates in eggs laid (mosquitoes tested singly)

Experimental period	No. of Mosquitoes	Test mosquitoes			Controls		
		Eggs laid	Eggs hatched	% eggs hatched	Eggs laid	Eggs hatched	% eggs hatched
up to 6 hrs.	22	1218	0	0	1677	1287	77
6 - 8 hrs.	8	405	34	8	675	561	83
8 - 10 hrs.	6	343	37	11	392	269	69
10 - 12 hrs.	6	332	146	44	509	431	85
over 12 hrs.	10	530	265	50	862	718	83

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

MR. W. H. CHEONG
(Division of Entomology, IMR, Kuala Lumpur)

Confirmation of DDT and Dieldrin Resistance and malathion sensitivity in **Cimex hemipterus**

From time to time reports have been received from various parts of Malaysia that bed-bugs were resistant to insecticides in use. These insecticides were DDT, BHC and Dieldrin. Following these complaints, Reid[1] carried out a laboratory investigation on one strain of the reported resistant bugs collected from a tea-estate on the coast of Selangor on which BHC and Dieldrin have been officially used, the most recent of which was dieldrin. The test insecticide was malathion, an organophosphorus compound, which had given good results against mosquitoes previously. (Reid and Chee[2]). The bugs were reared in the laboratory and fed on guinea-pigs until there were sufficient adults for testing by the method based on Busvine[3]. Reid found that the bugs were highly resistant to dieldrin and young hatched out during the test survived; in tubes containing malathion, except the control, they died. There was also a marked tolerance for DDT.

Following complaints from a labourers' quarters in the Kuala Lumpur area where DDT has been sprayed irregularly, the last occasion being a few months prior to complaints, a test was carried out. The tenants were either Malays or Indians. There were two blocks of ten houses each and another of four houses. Nineteen of these houses were inspected and bugs were collected from eleven of them. The test method was exactly the same as that of Reid[1] based on Busvine[3]; that is, using test-tubes with ready-prepared W.H.O. test papers for DDT and dieldrin and malathion paper prepared here. The papers 2 cm x 5 cm were folded once lengthwise.

The bugs were fed 48 hours before the test. The test was carried out on an exposure of 120 hours (5 days) at normal room temperatures with daily records of numbers dead. The tables show the results recorded with that of Reid.

TABLE I. TEA ESTATE

Test Paper	%	No. of bugs dead after 5 days
Control	0%	0/30
DDT	4%	10/20
DDT	8%	18/20
Dieldrin	1.6%	1/20
Malathion	0.01%	6/25
Malathion	0.02%	21/25
Malathion	0.05%	25/25
Malathion	0.1%	15/15

TABLE II. KUALA LUMPUR

Test Paper	%	Number of bugs dead after 5 days	8 days	12 days
Control	0%	1/15	1/15	1/15
DDT	4%	4/15	6/15	11/15
DDT	8%	2/15	6/15	12/15
Dieldrin	0.8%	2/15	6/15	7/15
Dieldrin	1.6%	2/15	6/15	9/15
Malathion	0.01%	10/15	13/15	15/15
Malathion	0.02%	14/15	15/15	15/15
Malathion	0.05%	15/15	15/15	15/15
Malathion	0.1%	15/15	15/15	15/15

At a glance both tables show fairly similar results except for minor differences. There was a definite resistance to both DDT and dieldrin in the Kuala Lumpur strain for Busvine gives a normal LD50 of dieldrin for *C. lectularius* at 0.07% and of DDT at 1.2%. With 1.6% dieldrin 26.7% of the bugs were killed in five days and with half the amount (0.8%) dieldrin, 13.3% mortality was recorded. Even after

twelve days of exposure, 60% and 46.7% had been killed respectively for the two concentrations.

The next insecticide DDT at 8% concentration gave a kill of 13.3% and at 4% concentration a kill of 26.7%. These clearly showed resistance and it was observed that the newly hatched bugs survived through from the seventh to the twelfth day. The Kuala Lumpur bugs seem to be more sensitive than the tea-estate bugs to malathion; the LD50 being slightly less than 0.01% whereas that of the tea-estate was slightly more than 0.01%. By the twelfth day all bugs in contact with malathion were dead.

It would appear that *Cimex hemipterus* readily acquires resistance to DDT and dieldrin in places where these are used in Malaysia. The experience of the Malaria Pilot Eradication Project at Kuala Selangor where this insecticide is used is a good example. It would therefore be important that steps should be taken to solve the problem which could do a lot of harm in any major campaign.

REFERENCES:

Reid, J. A. (1960). **Bull. Wld. Hlth. Org., 22,** 586-87.

Reid J. A. and Chee S. L. (1959). **Med. J. Malaya, 13**, 239-42.

Busvine, J. R. (1958). **Bull. Wld. Hlth. Org., 19,** 1041-52.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. DAVID W. ELLISON and COL. HINTON J. BAKER
(U.S. Army Medical Research Unit, IMR, Kuala Lumpur)

Pseudomonas pseudomallei from the Gombak River

The presence of melioidosis in Malaya has been known since 1913. Most instances of recorded isolations of *Ps. pseudomallei* in this country have been from either infected animals or man. Clinically, this disease is rare, easily confused with other maladies, and quite often fatal with the primary gross lesions being those of multiple abcesses in the lungs, liver, and spleen. Many of the survivors in whom this disease has been diagnosed exhibit draining suppurative lesions, abcesssed lymph nodes or swollen joints. The organism has been isolated from nature in other countries. Having been found in stagnant water, ponds, and rice swamps in Vietnam, melioidosis was described as "hydrotelluric" disease. An excellent review of the work done on the natural occurence of the organism in nature by several French workers in Vietnam has been presented by Rubin.

By using hamster inoculation techniques developed by this laboratory and standard cultural procedures, seven isolates were recovered from water samples and susequently confirmed as being *Ps. pseudomallei* by the Walter Reed Army Institute of Research in Washington, D.C. All isolates were recovered from flowing water from five different sites on two different days. One isolate was obtained from the first site, a jungle stream 16 miles from Kuala Lumpur that flows into the Gombak River. Another isolate came from the main river about 7 miles from town. Two isolates were made from a branch of the main river 6¾ miles from town just before it enters some rice fields. Three other isolates came from the effluent of the some water after it had flowed through the rice fields some 6 miles from town.

Subsequent isolates, though not yet confirmed, indicate further contamination of these same sites plus certain stagnant pools of water around the Kuala Lumpur area.

Further investigations will be conducted to try to determine the sources of the contamination.

In view of the fact that this organism is found in the waters frequented by man and animals, both veterinary and medical officers should certainly consider this disease when trying to make a diagnosis and should stress to their contacts the importance of utilizing a sanitary water supply.

Cultures of *Ps. pseudomallei* on differential media were demonstrated.

REFERENCES:

Joubert, L. and Phung Van Dan (1958). Epidemiology and prophylaxis of melioidosis in man and animals in the tropics, **Rev. Elev.** 11, 23-29.

Lee Chin Hua (1961). A Note on melioidosis in a serow in Perak, **Malayan Vet. Med. Assn.,** volume III No. 2.

Omar, R.A., Cheah Kok Kheong, and Mahendranathan, T. (1962). Observations on porcine melioidosis in Malaya, **Brit. Vet. J. 118,** 421.

Retnasabapathy, A. (1959). Melioidosis in pigs, **Malayan Vet. Med. Assn.,** volume II, No. 3.

Rubin, H. L.; Alexander, A. D.; and Yager, R. H. (1963). Melioidosis — A military medical problem? **Military Medicine,** vol. 128, No. 6.

Shanta, C. S. (1960). A note on the isolation of **Pf. whitmori** from the large intestines of a goat, **Malayan Vet. Med. Assn.,** volume III, No. 1.

Stanton, A. T. and Fletcher, W. (1932). **Melioidosis,** Studies of I.M.R. No. 21.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

MR. CHARLES F. NEEDY
(USAMRU, IMR, Kuala Lumpur)

The Indirect Fluorescent Antibody Technique in Scrub Typhus Studies

Indirect FA staining (1) as used in this laboratory is modified Coombs type of reaction in which the binding of unlabeled antibody to antigen is visualized by means of a second stage "FA indicator rather than by "second stage" serologic reactions such as agglutination. The indirect test allows the investigator to determine either the anitbody level of an unknown serum or the identity of an unknown antigen. (2) The FA technique as employed by Goldman (3) in 1954 suggested the potential application in other fields. The usefullness of this tool as a rapid diagnosis of scrub typhus (4) is hereby described.

METHODS AND MATERIALS

(a) Impression smears of mouse peritoneum infected with the Karp strain of R. orientalis (Scrub typhus) were air dried, fixed 10 min. in acetone, air dried and stored at-65° C. These smears can be held at least 3 months at this temperature and are considered as the antigen source.

(b) Three areas of the infected smear are ringed with finegrnail polish, the first area designated the test smear: the second and the third are used as negative and positive controls, respectively.

(c) The test smear is covered with undiluted, unknown serum from the patient whose antibody is being tested. The negative control smear is covered with undiluted, known negative serum. The positive control is covered with undiluted, known positive serum.

(d) Slides are incubated for 30 min. in a moist chamber (plastic slide box lined with wet filter paper) at 37° C.

(e) Slides are rinsed two times for 5 min. each with phosphate buffered saline pH 7.2 followed by a quick rinse in distilled water.

(f) Slides are air dried (cool) by means of a hair dryer.

(g) All smears are covered with Fluorescein Isothiocyanate labeled anti-human globulin (horse origin).

(h) Step d, e, and f are repeated.

(i) Slides are mounted in buffered-glycerol saline and examined.

Approximate time involved to label product is ca. two and one half hours.

Slides are examined at a total magnification of X 516 with a fluorescence microscope equipped with a high-pressure mercury-vapour lamp and dark-ground condenser of a numerical aperature 0.80. Excitation with blue fluorescence (i.e. U.V. + blue) was provided by a Leitz BG 12 primary filter and a blue absorbing filter was placed in the ocular.

DISCUSSION

Fluorescence indicates the presence of homologous antibody in the test serum. Unlabeled antibody (i.e., human serum) plays a dual role, acting as: (A) antibody in the primary reaction (step c) and, (B) antigen in the secondary reaction (step g). (2) In 20 sera, all negative for OX-K antibody using the Weil-Felix Reaction, 6 proved positive by this method.

REFERENCES:

Weller, T. H., and Coons, A. H. (1954). Flourescent antibody studies with agents of varicella and herpes zoster propagated in vitro. **Proc. Soc. exp. Biol. Med. 86:** 789-794.

Cherry, W. B., Goldman, M., Carski, T. R. and Moody, M. D. (1960). Fluorescent antibody techniques in the diagnosis of communicable diseases. **U.S. Pub. Hlth. Service Publication,** No. 729.

Goldman, M. (1954). Use of fluorescein-tagged antibody to identify cultures of Endamoeba histolytica and Endamoeba coli. **Amer. J. Hyg. 59:** 318-325.

Bozeman, F. M. and Elisberg, B. L. (1963). Serological diagnosis of scrub typhus by indirect immunofluorescense. **Proc. Soc. exp. Biol. Med. 112:** 568-573.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. DORA TAN
(Division of Virus Research and Medical Zoology, IMR., Kuala Lumpur)

Sellers' Technique
A rapid microscopical examination for Negri bodies in the diagnosis of rabies

In the Laboratory diagnosis of rabies it is important that the techniques employed should be accurate, fast and economical. The method employing the microscopical examination for Negri bodies, using the simple application of brain tissue to a slide and Sellers' technique for staining, has been proved to fulfil these requirements. During the recent outbreak of rabies in Malaya, much was owed to this technique for the prompt and effective control of the spread of the disease throughout the country.

As it has been found that Negri bodies, when present, are most readily demonstarted in Ammon's horn (hippocampus major) of the brain and also in the pyramidal cells of the cerebral cortex and Purkinje's cells of the cerebellum, these portions of the brain tissue are usually examined.

Three methods of applying fresh brain tissue to slides are recommended: impression method, "roling" method and smear method. We prefer the last one as in this technique there is a copious concentration of tissue and a rather extensive area for examination.

Sellers' stain shows the Negri body well-differentiated in magenta to bright red, with well-demonstrated dark-blue to black basophilic inner bodies. All parts of the nerve cell stain blue, and the interstitial tissue stains pink. Erythrocytes stain copper-colour (orange-tinged red) and can be easily differentiated from the magenta-tinged red of the Negri bodies.

The best results with the stain are obtained when the brain tissue is fresh. As decomposition sets in, the characteristic colour differentiation is affected and although the Negri bodies retain their staining quality, the smear as a whole becomes too red, or at times too blue, and identification of the bodies becomes more difficult.

THE NEGRI BODY

Although generally rounded in form, the Negri body may be found to assume any shape. It has been demonstrated to be round, oval, spheroid, amoeboid, elongate, triangular, etc. There is also great variation in size; generally it is found within the limits of 0.24u to 27.0u. It is characteristically acidophilic in staining reaction, and takes on the pink to purplish-pink colour in differential stains which use basic fuchsin or eosin with methylene blue as their base.

The position of the Negri body within the neuron is intracytoplasmic. However, this position can be expected only in histological sections of the brain. In the simple tissue-application techniques described above, the histological pattern is disturbed and one may very often see well-formed Negri bodies which appear to be entirely outside the neuron.

The most characteristic feature of the Negri body is its internal structure. It is this feature which serves as the most essential criterion for positive identification. The matrix of the Negri body has an acidophilic staining reaction, and contained within this magenta-red structure are small inner bodies (Inner-körperchen), basophilic granules which stain dark-blue to black. The size of these inner granules generally varies from 0.2u to 0.5u. Classically, the well-formed Negri body — the so-called

text-book picture — will have its inner granules arranged in rosette fashion, with one large centrally-placed body and a series of smaller granules arranged neatly around the periphery of the Negri body. However, this picture is the exception rather than the rule, and it is very rare indeed that such an orderly arrangement of the inner granules is seen.

The Medical Journal of Malaya
Vol. XIX. No. 1. September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. NYVEN J. MARCHETTE
(University of California International Center, for Medical Research, IMR, Kuala Lumpur)

Gal Filtration

Gel filtration is a simple method for separating water soluble materials differing in molecular size. In practice the filtration is done through a bed of packed grains of an inert hydrophilic material whose long carbon chains are cross-linked to form an open mesh-like structure.

The gel column on display was prepared from a granular dextran (Sephadex G-25 (R)) with a moderate degree of cross-linkage giving a gel of medium porosity when suspended in aqueous solution. Each dextran grain is a three-dimensional network of cross-linked polysaccharide chains. They are insoluble in ordinary aqueous solutions and are strongly hydrophilic. When placed in water, the grains swell forming gel grains; the degree of swelling being determined by the degree of cross-linking of the polysaccharide chains. The degree of cross-linking in the dextran grains also determines the size of the molecules which can diffuse into the gel grain. Solutes of sufficinetly low molecular size can diffuse relatively freely through the network structure in the grains, but large molecules are completely excluded. Thus it is readily seen how water soluble substances of large molecular size can be separated from solutes of small molecular size.

A useful application of gel filtration is in the purification of serum globulins after fractionation from whole serum with ammonium sulfate. The ammonium sulfate can be removed by dialysis against distilled water or a buffer, but this may take hours or days. It can be accomplished much more rapidly by gel filtration. In the latter method, the small ammonium sulfate molecules readily diffuse into the gel grains, but the large globulin molecules are completely excluded and flow through the aqueous matrix existing between the gel grains, and are collected at the bottom of the column. After all the pure globulin has come off the column, the ammonium sulfate molecules can be "flushed" from the gel grains with an excess of distilled water, saline or buffer and the column is speedily regenerated and ready for reuse.

Other applications of gel filtration are:

- separation and purification of enzymes and cofactors
- concentrating solutes of high molecular weight
- purification of haptoglobin
- purification of allergens
- separation of fluorescent antisera and unconjugated dye.

(R) Sephadex is the trade mark of Pharmacia, Uppsala, Sweden.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. NYVEN J. MARCHETTE
(University of California International Center, for Medical Research, IMR, Kuala Lumpur)

Semi-micro complement fixation test

The development of semi-micro serological methods has made it possible to conduct extensive serological surveys which would be technically or economically impractical using conventional macroserological methods. The micro titer kit is one of the gadgets developed to make efficient use of some of these methods. Extensive use for over five years in hundreds of laboratories has shown it to be quite reliable and to produce results which are comparable to those obtained by conventional macro-methods. The major advantage of using this instrument is the great economy of time and materials which can be realized without sacrificing accuracy and reliability.

The micro-titer kit on display here has been in almost constant use for over a year in the IMR Virus laboratory. Approximately 12,000 complement fixation tests for rickettsial antibody have been conducted requiring approximately 120 ml of CF antigens. If the conventional CF tube test had been done, 10 times that amount (or 1,200 ml) of antigen would have been required for the same number of tests. The saving in antigen alone is considerable. Of even greater importance, however, is the small amount of serum required for semi-micro serological tests. A minimum of three CF tests can be run on 0.1 ml of serum.

In practice the entire complement fixation test is conducted on a semi-micro scale using this kit. The preliminary hemolysin and complement titrations as well as all the controls and the serum anti-body titrations are done in the microtiter plates. The amounts of serum and reagents used are: diluent- 0.025 ml; serum- 0.025 ml; antigen- 0.025 ml; complement- 0.050 ml; haemolysin- 0.025 ml; sheep red blood cells- 0.025 ml.

The Medical Journal of Malaya
Vol. XIX. No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. JAMES CHIN
(University of California International Center for Medical Research, IMR, Kuala Lumpur)

Haptoglobin and Transferrin Studies in Malaysia

During the last two years, genetic surveys have been conducted from the IMR, Kuala Lumpur, in selected population groups of Malaysia. The objectives of these surveys are to establish baseline frequencies for several genetic factors in the different groups under study and to attempt correlation of some of these genetic factors to disease entities. One study currently in progress is a Haptoglobin (Hp) and Transferrin (Tf) survey of different racial groups in Malaysia. Since the initial publications of Smithies establishing the genetics of these serum proteins, great attention has been focused on them.

The method employed to study these genetically controlled serum factors is starch gel electrophoresis. This method for the separation of protein fractions essentially depend on the combination of electrophoresis and ultrafiltration (the gel as the supporting medium also serves as a fine mesh filter). For the detection of Haptoglobins in starch gel, use is made of their physiological function. After in vivo hemolysis every effort is made to conserve the body's iron supplies, and one of the mechanisms is through the complexing of free hemoglobin with circulating Haptoglobin (the 2-globulin). Prior to electro-phoresis of serum for Hp and Tf determination, free hemoglobin is added to the serum in vitro, in sufficient amount to saturate the hemaglobin binding capacity of the Haptoglobins. After electrophoresis, the gel is sliced in half and one of the halves is stained with a benzidine reagent to detect the peroxidase activity of the Haptoglobin-hemoglobin complex.

Four main Haptoglobin types have been identified by starch gel electrophoresis of serum, according to the number of molecules of hemoglobin bound and the mobility of the Hp-Hemoglobin complexes. They are referred to as Hp 1-1, Hp 2-2, Hp 2-1, and Hp 0-0. These Hp types are controlled by a pair of alleles; the Hp 1 gene and the Hp 2 gene (the 0-0 type has not definitely been proven to be genetically controlled).

The work of Kirk et al. (1960) demonstrated the frequency of the Hp 1 gene to be 0.24 in Malayan Malays. The present survey of Hp types in Malaysia seem to support this general frequency for Malayan Malays, but of interest is that Malays from Brunei have a significantly higher frequency of the Hp 1 gene. The reason for this difference is not immediately evident.

Transferrins, the other genetically controlled serum protein under study also has a very important physiological function. It is the iron-binding protein which transports iron to the iron stores and to the marrow. At present, there appears to be at least a dozen autosomal genes occupying a single locus on homologous chromosomes. By far the most common Transferrin gene is Tf^c, and most people are homologous for this gene. The other Transferrin types are named in accordance with their mobility rate in starch gel. Thus, those that migrate faster than Transferrin C are called B1, B2, etc., and those that migrate less rapidly are called D1 etc. Transferrins can be identified on the same gel that is used for Haptoglobin determination. After electrophoresis, half of the gel is stained for Haptoglobin and the other half is stained for general proteins. The pattern of the Transferrins can then be seen as the B1-globulin, between the Hp 1 band and the free excess hemoglobin.

Work with Transferrin identification in Malaya has just started and not much can be reported at this time except that Transferrin types other than C, probably subtypes of D are present in Brunei Malays.

REFERENCES

Smithies, O. (1955). Zone Electrophoresis in Starch Gel. **Biochem. J.**, 61:629.

Kirk, R. L., Lai, Y. Y. C., Mahmood, S., and Singh, R. B. (1960). Haptoglobin Types in S. E. Asia. **Nature, Lond.**, 185:185.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. LIE-INJO LUAN ENG
(Division of Nutrition (Haematology), IMR, Kuala Lumpur)

Alkaline phosphatase Activity in Leucocytes of Animals

The study of the enzyme alkaline phosphatase in neutrophilic leucocytes has become a useful tool in differential diagnosis in clinical medicine. The activity is increased in different conditions such as certain infections, malignancy and pregnancy. It is decreased in leukaemia. Especially in the tropics where concealed infections are prevalent, this study is expected to be a valuable aid.

Kaplow (1955) described a histo-chemical method for demonstrating this activity in the plasma of neutrophilic leucocytes. By this staining method a cell with enzyme activity is stained brown. The greater the enzyme activity, the darker the staining of the plasma. The activity is rated in every cell as 1+, 2+, 3+ and 4+. When 100 cells are counted the total number of the ratings is called the score.

Lie-Injo and Govindasamy found in 53 normal healthy Malayans a mean score of 19.3 with a range of 3 to 65, while in 116 newborns and 240 young infants below 1 year they found the activity to be physiologically much increased. With this procedure, it is essential to stain a positive control along with the unknown and it has always been a problem to find a positive control. We have examined different species of laboratory animals in the hope of finding species with a physiologically constant and definite increase of alkaline phosphatase activity in the neutrophilic leucocytes. It was found that normal heathy adult guineapigs and hamsters have a very high alkaline phosphatase activity in the leucocytes (slide 1). The score in 20 hamsters and 20 guineapigs was found to be above 200 in everyone of them. The leucocytes of mice however, are devoid of alkaline phosphatase activity (slide 2). Also in adult rabbits and rats the activity is increased when compared with normal healthy persons. With this finding the problem of having available positive controls in the laboratory is solved. While blood of hamsters and guineapigs can serve as a positive control, that of mice can be used as a negative control. Of more fundamental importance is, of course, the demonstration herewith that the metabolism involving the enzyme alkaline phosphatase is different in different animals.

REFERENCE

Kaplow, L. S. (1955). **Blood**, 10, 1023.

The Medical Journal of Malaya
Vol. XIX, No. 1, September 1964.

LABORATORY MEETING

held under the auspices of the ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE on January 10, 1964 at the INSTITUTE FOR MEDICAL RESEARCH, KUALA LUMPUR

DR. ANTHONY BALFOUR
(Pathologist, Royal Air Force Hospital, Changi, Singapore)

and

DR. IVAN POLUNIN
(Reader, Department of Social Medicine and Public Health
University of Singapore)

Some Haematological Data from a Laotian Village

The Village of Ban Na Khoun Noi lies about twelve miles north-east of the capital of Laos, Vientiane; it appears slightly more prosperous than most Lao villages, and is probably affected by the nearness of the capital. The people are Lao (Laotians), a race similar to the Thai. Their staple food is glutinous rice. There is not a great deal of animal food to be had, but mammals, birds, reptiles, amphibia and insects of many species are eaten; and also leaves, roots, fruits, and seeds of many species of wild, semi-wild and cultivated plants, but not in very great quantities. The climate is monsoonal, with well defined wet and dry seasons.

Specimens of venous blood were taken from the twenty-three adult villagers willing to be bled on the first of a series of visits, citrated, and kept on ice. The tests were done seventy-two hours after the samples were taken instead of the expected twenty-four hours, owing to unforseeable difficulties in transit. Most of the specimens appeared to be in good condition when tested in spite of the delay; but Nos. 5, 9, 10, 11, 12, 16 and 18 showed varying degrees of haemolysis, and there were some clots in Nos. 12 and 23. The results are given below in Table I; obviously worthless results have been omitted, and suspect figures are given in brackets. Comments on the findings are given in the next paragraphs.

Blood Groups

Group O	39%	(9 of 23 samples)
Group B	26%	(6 of 23 samples)
Group A	22%	(5 of 23 samples)
Group AB	13%	(3 of 23 samples)

Even in this small series, the B antigen is present in nearly 40% of the samples, emphasising its frequency in this part of the world.

Plasma Proteins (determined by the Copper Sulphate method) ranged from 6.2 to 7.2, with an average of 6.9 G/100 ml. Only No. 1 fell below the normal range given by Whitby (1963) of 6.6 to 8.1; and this range probably applies to Englishmen on a diet which is likely to be richer in protein.

Erythrocyte Sedimentation Rates (Wintrobe's method, corrected for packed cell volume) are unlikely to be reliable on such old samples; but 74% were within the normal range, 18% doubtful; and only 4% i.e. 1 sample — moderately increased and 4% markedly increased, namely samples 19 and 20.

Haemoglobins (oxyhaemoglobin method) Taking 11G/100ml as the lowest acceptable limit of nomality, 60% of the series were within normal limits, and another 25% were over 9.0 G/100ml but showed moderate anaemia; and only 15% showed marked anaemia. These three samples are being investigated further. No. 4 is probably a case of iron deficiency; but Nos. 14 and 15, who belong to the same household, are suspected of having haemoglobinopathies on clinical grounds. Electrophoresis is being done to see whether there may be a heterozygous HbE or Thalassaemia, or whether the Hb is in fact normal.

Total Leucocyte Counts were mostly on the low side, only 10% being over 5,000 cells/mm; some cells may have been destroyed in transit,

but it seems likely that these results indicate a tendency towards mild leucopaenia, as is common in these climates. Three results were eliminated as suspect; of the others, 70% were over 2,000, 15% between 1,500 and 1,000, and another 15% between 1,500 and 2,000.

Differential Leucocyte Counts were pretty well within normal limits except for Nos. 8 and 21, which showed a reversal of the normal polymorphonuclear-mononuclear ratio. Several showed tendencies towards eosinophilia, but only two samples, Nos. 2 and 18, had absolute eosinophil levels above 250; and the highest was only 380, of very doubtful significance.

Parasites. No filaria or malarial parasites were seen in searches of thick films, or on thin films during the differential leucocyte counts.

A full account of various investigations on these and other samples is to be presented later; meanwhile we hope that this preliminary communication giving the results available now may be of interest, because so little is known about the haematology of Lao villagers at present.

No.	Blood Group	Plasma Protein	E.S.R.	P.C.V.	MCHC	Hb G%	Hb %	WBC	Differential P	Differential L	Differential M	WBC E
1.	A	6.2	3	39	31	14.6	101	4000	49	42	4	5
2.	AB	6.9	10	(30)	—	15.4	107	2800	56	29	5	10
3.	A	6.9	8	35	36	12.6	86	2600	51	39	4	6
4.	B	6.9	5	30	29	8.6	59	6200	68	29	0	3
5.	B	6.6	3	38	34	12.9	89	3300	46	48	1	5
6.	O	7.2	2	39	39	15.2	105	1700	55	41	2	2
7.	O	6.9	2	27	35	9.4	64	5400	64	34	2	0
8.	O	7.0	1	33	35	11.7	81	4100	34	60	2	4
9.	O	7.0	1	38	35	13.2	91	3500	68	25	2	5
10.	B	6.9	2	42	33	13.7	95	4200	54	40	2	4
11.	B	6.9	1	35	39	13.7	99	1800	57	35	3	5
12.	A	6.9	2	—	—	—	—	—	67	37	1	1
13.	B	7.0	3	30	32	9.7	67	1000	56	43	1	0
14.	A	7.2	2	25	31	7.7	53	3200	59	40	0	1
15.	AB	6.9	2	23	35	8.0	55	2000	67	29	2	2
16.	O	6.8	0	(20)	—	13.7	95	3300	64	27	2	7
17.	O	6.9	10	30	31	9.4	64	1300	41	55	4	0
18.	AB	7.0	2	35	31	10.9	75	2700	50	36	0	14
19.	O	7.0	30	39	33	12.9	89	2800	53	36	3	8
20.	A	7.0	27	35	29	10.0	69	1500	66	31	1	2
21.	A	7.0	1	32	35	11.2	77	1400	41	59	0	0
22.	O	6.8	10	25	34	(8.6)	(59)	(850)	58	39	1	2
23.	O	—	—	—	—	(8.9)	(61)	(950)	—	—	—	—

REFERENCE

Whitby, L. E. H., (1963). **Disorders of the Blood,** 9th edition, p. 793, London: J. & A. Churchill Ltd.