LABORATORY DEMONSTRATIONS

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THE USE OF THE LEUCOCYTE ADHERENCE INHIBITION TEST TO MEASURE CELL MEDIATED IMMUNITY IN *RICKETTSIA TSUTSUGAMUSHI* INFECTIONS

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The Leucocyte Adherence Inhibition test (LAI) has been used by Halliday and Miller (1972) to detect cell mediated immunity (CMI) to tumor antigens in mice. The test offers several advantages over the more commonly employed tests to measure CMI. (1) It is rapid requiring approximately $\frac{1}{2}$ day to complete. (2) It requires only common laboratory equipment: hemocytometers and microscopes. (3) It requires a small number of leucocytes compared to other common methods of detecting CMI. (4) No aseptic precautions are required. For these reasons the conduct of the test is within the scope of any laboratory.

Briefly, the test is based on the elaboration, by sensitized lymphocytes in the presence of antigen, of a lymphokine which interferes with the adherence of macrophages to glass surfaces. The test is conducted by incubating a washed, standardized suspension of peritoneal macrophages from the test animal with the test antigen in the presence of immune or normal sera. The appropriate control consists of the uninfected substrate used to grow the rickettsial suspension. In our case the Karp strain of *R. tsutsugamushi* was grown in yolk sacs of embryonated eggs, and the control material was uninfected yolk sacs processed in the same manner.

In the first set of experiments (Exp. 1, Table 1) the reactants were incubated in the presence of peritoneal macrophages from normal animals. The mean per cent adherence dropped from >80 in the presence of normal yolk sac with either immune or normal sera to 27 in the presence of antigen with immune sera. The smallest value was 10% found with antigen and normal sera. These values are similar to those reported by Halliday in that to a certain extent, immune serum blocks the response of the cells to the antigen. It is surprising that the reaction appeared to be measurable with cells from non-immune individuals.

When the LAI was repeated with immune mice (Exp. 2, Table 1) the results are compatible with the origin of the reagents. Adherence was inhibited most strongly when the cells were reacted with antigen in the presence of nonimmune sera. There is a significant increase in the number of cells adhering in the presence of antigen and immune sera as compared to antigen and nonimmune sera. This indicates that the reaction is specific in that it is inhibited by immune sera but not by normal sera.

Table 1

Leucocyte adherence inhibition by *R. tsutsugamushi* antigens.

Source of leucocytes	Antigen extract	Sera	Adherent cells (% of Total)
Experiment No. 1			
Normal	Karp	Normal	10
		Immune	27
Normal	Normal yolk sac	Normal	83
		Immune	87
Experiment No. 2			
Immune	Karp	Normal	5
		Immune	23
Immune	Normal yolk sac	Normal	17
		Immune	18

There was a wide difference between control values. This has also been noted by Halliday and Miller (1972), and they caution that percentages are not comparable from experiment to experiment. The conclusions are comparable; and in our experiments, Karp antigen was effective in interfering with the adherence with specific immune sera acting as a blocking agent. When the source of the cells was immune mice the adherence returned to control values. When the source of the cells was normal mice the adherence was significantly increased but did not approach control values.

These preliminary data tend to indicate that the LAI can be adapted to the study of infectious diseases. Nothing is known about the persistence of CMI following scrub typhus or the ability of different strains to elicit CMI. The possibility that strains may produce CMI which can be stimulated by unrelated strains is of the upmost importance. This simple technique shows promise of being a useful tool in the investigation of these parameters.

REFERENCE

HALLIDAY, W.J. and MILLER, S., (1972). Leukocyte adherence inhibition: A simple test for cell-mediated tumour immunity and serum blocking factors. *Int. J. Cancer.*, *9* : 477.

BABESIA INFECTION IN A TEN-DAY OLD CALF

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A ten-day old female Droughtmaster calf carcass was brought for post-mortem examination to the Regional Veterinary Diagnostic Laboratory, Petaling Jaya. It was one of the calves born to recently imported Droughtmaster cattle from Queensland, Australia to Malaysia.

Blood smears and organ smears from the spleen and brain as well as the small intestines were taken for parasitological examination.

The blood and brain smears stained with May-Grunwald/Giemsa stain showed large numbers of *Babesia argentina* parasites. Smears from the spleen, too, showed a fair number of *Babesia* organisms. No coccidia parasites were observed.

Although *Babesia* infections are fairly common in imported temperate breeds of cattle (Rajamanickam, 1970), attention is drawn to this case to show that very young calves can become infected and show signs of disease. The calf was only 10 days of age when it died and it is unusual to see a *Babesia* infection at such an age. It is known that natural immunity to Babesiosis is greater in young calves; however in this instance either the calf did not get colostrum early in its life or else the dam had never been exposed to Babesiosis before, and hence did not have colostral antibodies to Babesiosis. The incubation period for Babesiosis has been given as 8-15 days by various workers and it is therefore possible for this infection to be spread by cattle-ticks to new born calves in such a case.

The demonstrations shown were: a) *Babesia argentina* from a thin blood smear of dead calf. b) *B. argentina* in blood capillaries from brain of dead calf.

REFERENCE

RAJAMANICKAM, C., (1970). Blood protozoan diseases of imported temperate breeds of cattle in West Malaysia. *Kajian Veterinaire*, *2* : 145.

TRYPANOSOMES FROM A LARVA OF *BOOPHILUS MICROPLUS*

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While carrying out a survey to determine the infection rate with *Babesia bigemina* and *Babesia argentina* in cattle tick *Boophilus microplus*, we came across one larva, out of 101 larvae examined so far, having trypanosomes. Routine smears were made from the larvae and stained with Giemsa. These trypanosomes were seen in one of the smears.

The measurements of these trypanosomes were taken on five of them. These are given below in micrometers with averages in parenthesis.

Total length	22 - 31 (27.8)
width at nucleus	2.5 - 4 (2.9)
free flagellum	7 - 11 (9)

It is known that *T. evansi* is common among cattle and buffaloes in Malaysia. As this finding was accidental it was not possible to trace back the animal from which that particular larva was collected.

This finding is of interest in that there is very little information about the possibility of ticks playing a role in the transmission of trypanosomes. *Ornithodorus crossi* and *O. lahorensis* were able to transmit *T. evansi* (Cross, 1947). It had been shown that *T. cruzi* could develop in ticks, *Ornithodorus moubata* fed on infected mammals (Brumpt, 1912; Mayer and Rocha-Lima, 1914) and the infection in these ticks persisted for 5 years (Mayer, 1918).

REFERENCES

BRUMPT, E., (1912). Cited by Hoare C.A., In *The Trypanosomes of Mammals*, Blackwell Scientific Publications, Oxford and Edinburgh, 1972, pp. 358.

CROSS, (1947). Cited by Richardson U.F and Kendall S.B., In *Veterinary Protozoology*, Oliver and Boyd Ltd. Edinburgh, 3rd. ed., 1963, pp. 53.

MAYER, M., (1918). Cited by Hoare C.A., In *The Trypanosomes of Mammals*, Blackwell Scientific Publications, Oxford and Edinburgh, 1972, pp. 358.

MAYER, M. and ROCHA-LIMA, (1914). Cited by Hoare C.A., In *The Trypanosomes of Mammals*, Blackwell Scientific Publications, Oxford and Edinburgh, 1972, pp. 358.

FAILURE OF *BRUGIA MALAYI* AND *B. PAHANGI* TO DEVELOP IN NYMPHS OF *HAEMAPHYSALIS NADCHATRAMI* (ACARINA: IXODIDAE) FED ON EXPERIMENTALLY INFECTED HOSTS†

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In anticipation of investigating the vector potential of ticks for the rodent filarial worm, *Dunnifilaria ramachandrani* Mullin and Balasingam, of which the vector is unknown, familiarization with the pertinent techniques was acquired by observing the fate of

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Brugia malayi and *B. pahangi* in *Haemaphysalis nadchatrami* nymphs. Results of this limited study are summarized herein.

During May 1975, laboratory-reared nymphs were confined in capsules and fed on two domestic cats infected 7 and 14 months earlier with third stage larvae of *B. pahangi* and subperiodic *B. malayi*, respectively, harvested from *Aedes togoi* mosquitoes. Host diurnal parasitemia levels were determined at 4 hour intervals between 8 a.m and 8 p.m on the day that ticks were applied and on days 3 and 7 post application. Ticks were examined as replete or partially engorged nymphs on the day they detached or were removed manually and as unfed adults 2 to 6 days after ecdysis; nymphs held for molt were maintained at 25 ± 1°C and 98% RH. Tick tissues were dissected and gently macerated on glass slides in 0.85% or 0.35% saline.

A total of 26 microfilariae were observed in 7 of 26 (27%) nymphs exposed to *B. malayi*, and 38 microfilariae were observed in 5 of 25 (20%) nymphs exposed to *B. pahangi*. All of the parasites appeared to be dead and confined to the midgut. All of 22 adults exposed as nymphs to *B. malayi* were negative, whereas 9 parasites were found in 4 of 21 (19%) adult ticks fed as nymphs on the cat infected with *B. pahangi*. The latter parasites were dead, sheathed microfilariae, thus resembling the parasites observed in the nymphs. Development beyond the microfilarial stage was not seen. Photomicrographs of microfilariae recovered from the nymphs were demonstrated.

Although the inability of these mosquito-borne filariae to survive and develop in ticks was expected, the results of this study are qualified by our failure to examine the legs of the ticks for parasites. The possible importance of this oversight is supported by reports of live microfilariae of *Brugia patei* and sausage stage larvae of *B. malayi* in the legs of bedbugs previously fed on infected hosts (Nelson, 1963; Burton, 1962). The persistence (up to 16 days) of intact microfilariae in ticks that molted in the present study probably was related to the presence of these parasites in the midgut, the tissues of which are not greatly affected by metamorphosis. Ranges of microfilariae per 60 mm of host peripheral blood were: 585-2435 *(B. malayi)* and 665-1585 *(B. pahangi)*. By relating these data to the average nymphal blood meal size of 0.0048 ml (obtained by weighing 100 replete *H. nadchatrami* nymphs), it was estimated that each replete nymph would be expected to ingest approximately 42 to 195 or 53 to 127 microfilariae of *B. malayi* and *B. pahangi*, respectively. That the number of microfilariae seen in the ticks was well below the number expected may have been due either to our failure to examine the legs of the ticks or to the fact that *H. nadchatrami* is not an efficient vector of these parasites. Support for the latter view is provided by mosquito studies demonstrating that efficient vectors ingest more microfilariae and poor vectors ingest fewer microfilariae than would be expected on the basis of host parasitemia level and size of the mosquito blood meal (Wharton, 1957, 1962).

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REFERENCES

BURTON, G.J., (1962). Observations on filarial larvae in bedbugs feeding on human carriers of *Wuchereria bancrofti* and *Brugia malayi* in South India. *Amer. J. Trop. Med. Hyg., 11* : 68.

NELSON, G.S., (1963). Are bedbugs vectors of filariasis? *Trans. Roy. Soc. Trop. Med. Hyg., 57*: 149.

WHARTON, R.H., (1957). Studies on filariasis in Malaya: Observations on the development of *Wuchereria malayi* in *Mansonia* (Mansonoides) *longipalpus*. *Ann. Trop. Med. Parasitl., 51* : 278.

WHARTON, R.H., (1962). The biology of *Mansonia* mosquitoes in relation to the transmission of filariasis in Malaya. *Bull. Inst. Med. Res.* Malaya, No. 11, 114 p.

OCCURRENCE OF A SPOROCYST GENERATION OF *FASCIOLA GIGANTICA* IN THE SNAIL *LYMNAEA RUBIGINOSA*

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Ogambo-Ongoma and Goodman (1976) have recently reported the development of the miracidium of *Fasciola gigantica* Cobbold 1856 directly into a mother redia, bypassing entirely the sporocyst stage, in the snail *Lymnaea natalensis* Krauss 1848. Since this pattern of intramolluscan development differs substantially from that reported in previous studies, a brief investigation was conducted in order to confirm their observation.

All work was conducted at ambient temperature (25°C to 27°C). Eggs of *F. gigantica* were dissected from adult worms removed from the livers of cattle slaughtered at the Shah Alam abattoir, Kuala Lumpur, and were incubated in rainwater until hatching occurred. *L. rubiginosa* measuring 3 to 5 mm in shell length were exposed to 20 miracidia each and were dissected at 8 days post-exposure. According to Ogambo-Ongoma and Goodman (1976), 8-day old rediae had a thick body wall, a muscular pharynx, a distinct gut, and developing procruscula. In the present study, however, clearly recognizable sporocysts were obtained from the mantle collar of infected snails. These sporocysts contained motile mother rediae and germ balls. Sporocysts which were inadvertantly ruptured during dissection yielded young rediae, some of which closely resembled the 8-day old redia of Ogambo-Ongoma and Goodman (1976). However, no eyespots were observed in the rediae in the present study.

Since Ogambo-Ongoma and Goodman observed eyespots and miracidial flame cells in rediae and an exact correlation between the number of infecting miracidia and the number of mother rediae, their conclusion of a direct metamorphosis of miracidia into mother rediae is not questioned. However, in the present study, using specimens of *L. rubiginosa* and *F. gigantica* endemic to Malaysia, a sporocyst stage is unmistakably involved in the intramolluscan development, as has been described by Alicata (1938) in Hawaii, and Dinnik and Dinnik (1956) in East Africa. It

may be, therefore, that geographic strains of *F. gigantica*, or perhaps the same strain in different species of snails, undergo substantially different patterns of larval development.

REFERENCES

ALICATA, J.E., (1938). Observations on the life history of *Fasciola gigantica*, the common liver fluke of cattle in Hawaii, and the intermediate host, *Fossaria ollula*. *Hawaii Agric. Exp. Stn. Bull.*, *80* : 1.

DINNIK, J.A. and DINNIK, N.N., (1956). Observations on the succession of redial generations of *Fasciola gigantica* Cobbold in a snail host. *Z. Tropenmed. Parasit.*, *7* : 397.

OGAMBO-ONGOMA, A.H. and GOODMAN, J.D., (1976). *Fasciola gigantica* Cobbold 1856 in the snail. *J. Parasit.*, *62* : 33.

THE HAMSTER AS A LABORATORY HOST FOR *SCHISTOSOMA SPINDALE*

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Despite the suitability of the golden hamster, *Mesocricetus auratus*, as a laboratory host for *Schistosoma mansoni*, there have been no reports of *S. spindale* in the hamster. Since this mammal is more easily maintained than are the goat or calf, which are the common experimental definitive hosts for *S. spindale*, infection of hamsters with *S. spindale* was attempted.

From 250 to 1000 cercariae shed from *Indoplanorbis exustus* collected near Ampang Village, Selangor, were drawn up into a 5 ml hypodermic syringe and were injected into the peritoneal cavity of anaesthetized young adult hamsters. Faeces were examined weekly for the presence of eggs beginning at 6 to 7 weeks postinjection. Surviving hamsters were necropsied at 52 to 67 days postinjection. The excised livers were briefly macerated in a food blender, washed several times in 0.85% NaCl, and were transferred to rainwater in a one-liter volumetric flask which was exposed to light from a 60-watt bulb. The base and lower neck region of this flask had been painted black, leaving only the water in the upper neck region exposed to light. The water in this region was then examined for miracidia. In addition, liver squash preparations were examined microscopically for ova, and mesenteric and portal veins were inspected for adult worms.

Injections of 1000 cercariae proved fatal in both of 2 hamsters, while 500 cercariae effected approximately 50% mortality (5 of 11 hamsters) within 37 days. At the present time, 5 hamsters injected with 250 cercariae each and 2 hamsters injected with 500 cercariae each have been necropsied (Table 1).

Dutt (1962) has reported that females of *S. spindale* do not develop well in the guinea pig. In the present study, very few female adult worms were observed in the mesenteric and portal veins. The preponderance of males may be due either to the inability of females to develop in the hamster, or as is more likely, to the possibility that primarily male cercariae were injected, owing to the small number of shedding snails. Presumably the lack of female adult worms accounts for the low yield of miracidia. Specimens of *I. exustus* which were each exposed to 5 of these miracidia shed cercariae of *S. spindale* after 25 days.

Infection studies are underway with cercariae which have been shed from a larger number of naturally infected snails and from those experimentally infected with miracidia obtained from hamsters. Although the suitability of the hamster as a laboratory host for *S. spindale* remains in question, it has been shown that this parasite is able to complete its life cycle under laboratory conditions in *M. auratus*.

REFERENCES

DUTT, S.C., (1962). Studies on the susceptibility of the guinea-pig to infection with *Schistosoma spindale* Montgomery, 1906. *Parasitology*, *52*: 199.

Table 1

Infection of golden hamsters with *Schistosoma spindale*.

No. of cercariae injected	Days post-injection	Ova in faeces	Adults in blood vessels	Ova in liver squashes	No. of miracidia
250	52	None	Males and females, mostly males	Undeveloped	0
250	54	,,	,,	,,	,,
250	60	,,	,,	Undeveloped and developed	7
250	62	,,	,,	,,	9
250	67	,,	All males	None	0
500	56	,,	Males and females, mostly males	Undeveloped and developed	27
500	66	,,	All males	None	0