RESEARCH NOTES

EXPERIMENTAL INFECTIONS OF *CAPILLARIA PHILIPPINENSIS* IN MULTIMAMMATE RATS *(MASTOMYS NATALENSIS)*

The natural reservoir host for *Capillaria philippinensis* has remained an enigma since the parasite and disease, intestinal capillariasis, were first reported from the Philippines. A variety of animal specimens numbering in thousands have been examined in search for a natural host but adult worms have not been found except in humans. Experimentally, the parasite has been transmitted to monkeys (Cross *et al.*, 1972, *Trans. Roy. Soc. Trop. Med. Hyg.*, *66*: 819) Mongolian gerbils, and a few wild rats, (Cross *et al.*, 1978, *J. Parasitol.*, *64*: 208) by feeding the animals larvae from experimentally infected fish or by stomach tube passage of adult and larval worms from the intestines of infected gerbils (Cross *et al.*, 1972, *Trans. Roy. Soc. Trop. Med. Hyg.*, *66*: 819; Cross *et al.*, 1978, *J. Parasitol.*, *64*: 208; and Bhaibulaya *et al.*, 1979, *Int. J. Parasitol.*, *9* : 105). Bhaibulaya and Indra-Ngarm (1979, *Int. J. Parasitol.*, *9*: 320) have also reported experimental infections in fish-eating birds in Thailand and we have subsequently confirmed these findings by experimentally infecting fish-eating birds from Taiwan (Cross *et al.*, 1983, *Trans. Roy. Soc. Trop. Med. Hyg.*, in press).

The present communication is to briefly report experimental transmissions of the parasite to multimammate rats (*Mastomys natalensis*). Approximately 100 adult and larval parasites from gerbil intestines were introduced by stomach tube to five adult male multimammate rats and two Mongolian gerbils (*Meriones unquiculatus*) which served as controls. Two of the multimammate rats developed patent infections at 12 and 43 days and at necropsy at 43 and 76 days, 1775 and 195 adult and larval *C. philippinensis* were recovered from the rat intestines. No infections developed in two other rats and a single male worm was found in the remaining animal. One gerbil control was also negative, but in the other eggs were present in the feces at 12 days and when the animal was necropsied at 40 days 3,112 worms in all stages of development were recovered from the intestine.

Unfortunately, further studies could not be done but the preliminary findings indicate that another laboratory animal model is available for studies with *C. philippinensis*.

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THE USE OF CHLORAMPHENICOL IN THE CONTINUOUS CULTURE OF *PLASMODIUM FALCIPARUM*

The success of culturing *Plasmodium falciparum* continuously *in vitro* has made investigations possible in several fields where they were formally hindered by shortage of parasite materials. For example, studies on metabolism, chemotherapy and development of vaccines (Wernsdorfer, 1981, *Bull. WHO.*, *59*: 335) are progressing with the aid of continuous cultures. This long term cultivation allows observation on stability versus instability of *P. falciparum* and it elucidates variation of drug responsiveness among malarial parasite populations. For such studies, an isolate as well as cloned parasites must be cultured without interruption for over a year (Tan-ariya, 1982, Mahidol Ph.D. Thesis). This long term cultivation can be achieved only when problems of microbial contamination are solved.

In the past the antimicrobial of choice was gentamycin at a concentration of 40 μg/ml medium (Trager, pers. comm.). This antibiotic is suitable for culture work because it does not interfere with parasite growth and it is available as a sterile solution and can thus be mixed directly with the complete culture medium. However, we have found that gentamycin is effective only against coccal bacteria and not against the bacilli which are our major contaminant. This paper reports the use of chloramphenicol, a broad spectrum antibiotic in continuous culture.

Using the petri dish-candle jar system (Trager and Jensen, 1976. *Science*, *193*: 673) we cultured *P. falciparum* line FCM_5 in human erythrocytes group O in RPMI 1640 medium supplemented with 10% human serum. This complete culture medium was used to dilute a stock solution of 1% chloramphenicol sodium succinate in sterile water so that the final concentrations of the antibiotic were 12, 9, 6, 3, 0.6, 0.12 chloramphenicol/ml medium. Culture medium without drug served as a control. Two types of damage by the drug were tested (a) degree of erythrocyte lysis and (b) inhibition of parasite growth.

Non-infected erythrocytes were incubated with RPMI 1640 complete medium containing chloramphenicol at varying concentrations in a candle jar at 37°C for 24 hours. The degree of hemolysis was measured at 540 nm on a UNICAM spectrophotometer. Fig. 1 indicates that the erythrocytes were affected by chloramphenicol at a concentration of 3 mg/ml.

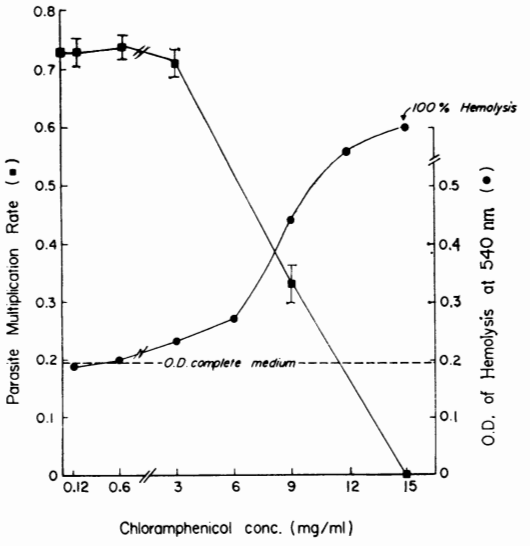

Fig. 1

Parasite development and thus growth of the population appeared to correlate conversely with the degree of hemolysis. At concentrations below 3 mg/ml the ring stage of *P. falciparum* could complete schizogony resulting in an increase in population size. During the four days of cultivation parasite multiplication rates (Brockelman *et al.*, 1981,

Bull. WHO., 59: 249) determined from 4 replicate cultures in the groups which received 0.12 mg and 0.6 mg of chloramphenicol per ml of medium were 0.72±0.03 and 0.73±0.05, respectively. Factorial analysis revealed that there were no significant differences in parasite multiplication rates when compared with the control group receiving no antibiotic.

Efficacy of chloramphenicol was also satisfactory in cultures which were readily contaminated with either coccal or rod shaped bacteria or both. Light contamination was detected microscopically on thin blood smears of culture materials stained with Giemsa. We treated such cultures by transferring each of them to a sterile screw capped centrifuge tube and then diluting 1 : 10 v/v with RPMI medium containing 0.2 mg chloramphenicol per ml. After two hours of incubation at 37°C the supernatant was removed and the settled erythrocytes were re-examined for bacteria. We found that chloramphenicol inhibited bacterial growth efficiently if the bacterial population did not exceed 20 cells per 1,000 erythrocytes. These cultures were kept under 0.05 mg chloramphenicol for the following two days to ensure complete cessation of bacterial growth.

Our experimental results reported here are in agreement with Schnell and Siddiqui (1972, *Proc. Helminthol. Soc. Wash., 39* : 201) who detected no inhibition on amino acid uptake by *P. falciparum* in the presence of 3×10^{-4} mol/liter chloramphenicol (0.969 mg/ml). Although Sherman *et al.*, (1971, *Int. J. Biochem., 2*: 27) found that 90% of the amino acid uptake by *P. lophurae* was inhibited by chloramphenicol at a concentration of 3×10^{-3} mol/liter (9.69 mg/ml) we believe that their drug concentration was too high. At that concentration we could readily detect lysis of erythrocytes and this could indirectly affect the malarial parasites. Hence, we conclude that chloramphenicol is a safe and potent antibiotic to be used in continuous culture of *P. falciparum* since it does not exert any inhibitory effect on the parasite. The protein ribosomal enzyme of *P. falciparum* is not a target of action of chloramphenicol as is that of prokaryotes. It is apparent that the plasmodial protein synthesizing system is typically eukaryotic.

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CREEPING ERUPTION CAUSED BY *GNATHOSTOMA SPINIGERUM*

Clinical manifestations of gnathostomiasis have been classified into cutaneous and visceral types (Daengsvang, 1980, SEAMIC Publication, N : 21 : p. 43). The cutaneous type presented swelling with either pain or itching or both. The visceral type presented manifestations depending upon the organs involved which could cause fatality. The morbidity of human gnathostomiasis was caused by migrating larvae. It was shown that larva of *Gn. spinigerum* was able to move as far as 3.0 cm. in an hour (Daengsvang, 1970. *Ann. Trop. Med. Parasit., 64* : 399). However, the occurrence of cutaneous type was observed to be more common than that of the visceral type.

Creeping eruption which was proved to cause by *Gn. spinigerum* has been reported, so far by Tamura (1921, *Brit. J. Dermatol.*

Syphil., 33 : 81) and Pinkus *et al.*, (1981. *Int. J. Dermatol., 20* : 46). Although Thailand is regarded as an endemic area of gnathostomiasis the creeping eruption caused by *Gn. spinigerum* has not been reported. A case of creeping eruption caused by this worm is now reported. A 30-year-old Thai female came to the hospital with the chief complaint of having tortuous eruption on her left thigh above the knee joint for 4-5 days. Twenty days before the onset of the eruption she ate raw fermenting salted freshwater fish locally called "Som Fak" and one week later she noticed a swelling with slight pain and itching at the right costal margin. The swelling disappeared within 2-3 days and reappeared at the left flank and left foot respectively. Six to seven days before coming to the hospital the eruption started at the anterior surface of left thigh above the knee joint and then extended serpentinously to the posterior surface. On examination it was found that the serpentinous eruption occurred around the left thigh above the knee joint (Fig. 1). The eruption at anterior surface of the thigh was rather brown in colour whereas at the posterior surface it was still slightly red with itching sensation. At the end of the creeping eruption tract at the posterior surface a black spot like-object was noticed

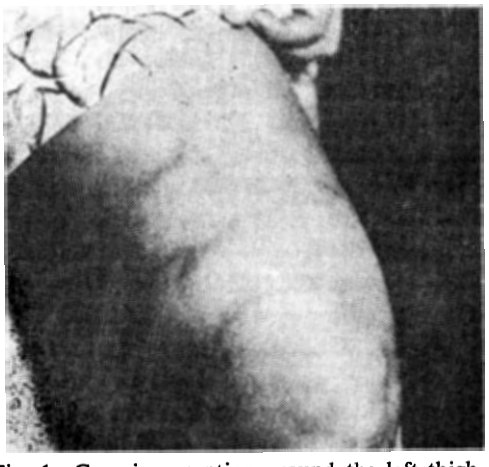

Fig. 1—Creeping eruption around the left thigh.

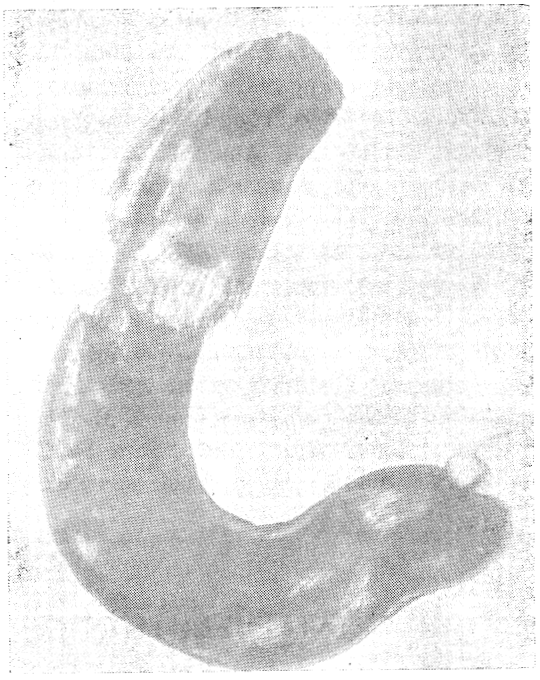

Fig. 2—Early third stage larva of *Gn. spinigerum* recovered from the end of creeping eruption × 75.

Fig. 3—Anterior end of *Gn. spinigerum* larva recovered showing 4 rows of cephalic spines × 300.

underneath the skin. The excision was made and an object of grey colour was obtained. On examination under the microscope it was found to be a round worm which measured 2 mm long and 0.5 mm wide. The anterior end possessed cephalic bulb with two lips. The cephalic bulb bore four transverse rows of spines. The body was also covered with transverse rows of minute spines. The internal organs seen were only the alimentary tract and the four-cervical sac. The worm was identified as the early third stage larva of *Gn. spinigerum* (Fig. 2, 3). The history of recent eating raw "Som Fak", migratory swelling and a black spot like object underneath the skin at the end of the creeping eruption tract suggested that the causative agent was *Gn. spinigerum*. From the observations, the worm was easily extracted when the black spot like object was seen underneath the skin. This is the first case of creeping eruption caused by *Gn. spinigerum* reported from Thailand.

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FILARIAL INFECTION OF THE EYE: A CASE REPORT

Filarial parasites may sometimes be found in abnormal locations in the human host. Developing or adult *Dirofilaria* sp. have been recovered from the eye and surroundings (Forbes, 1918. *Trans. Roy. Soc. Trop. Med. Hyg., 12* : 11; Faust, 1957. *Z. Tropenmed. Parasit., 8* : 59). Intraocular infections with *Wuchereria bancrofti* (Wright, 1934. *Brit. J. Ophthal., 18* : 646; Fernando, 1935, *J. Trop. Med., 38* : 17), possibly *B. malayi* (Rose, 1966. *Arch. Ophthal., 75* : 13) and *Dirofilaria immitis* (Dissanaike *et al.*, 1977. *Amer. J. Trop. Med. Hyg., 26* : 1143) have been described. Two cases of *Brugia* sp. infection of the conjunctiva were previously reported from Malaysia (Mak *et al.*, 1974. *Southeast Asian J. Trop. Med. Pub. Hlth., 5* : 226; Dissanaike *et al.*, 1974. *Amer. J. Trop. Med. Hyg., 23* : 1023). A further case of *Brugia* infection of the human eye is now reported.

L.W.S. a 29-year-old Chinese male, timber merchant was seen by one of us (Q.C.H.) complaining of a persistent red, irritable right eye for the past 6 weeks. On examination there was episcleritis at the lateral limbus where coils of a live worm were seen in a cyst-like structure measuring 0.5 × 1 cm. There was no other significant finding. The cyst-like structure was removed under local anesthesia and fixed in 70% alcohol. The worm, damaged during extraction, was removed and transferred to a mixture of 5% glycerine in 70% alcohol. It was then slowly brought into pure glycerine through evaporation for morphological studies.

The specimen consisted of the anterior portion of a nongravid female worm. The total length was 21.50 mm. and the maximum width was 0.11 mm. The head had the typically angulated, globular shape of *Brugia* sp. measuring 32 μm wide × 20 μm long (Fig. 1a, b), unlike that of the more rounded end of *W. bancrofti* (Fig. 1c). The nerve ring and vulval opening were 0.16 mm. and 0.44 mm from the anterior end respectively. The oesophagus, divided into anterior muscular and posterior glandular regions, measured 1.37 mm. The morphology and measurements corresponds very closely to that of an immature *B. malayi* (Rao and Maplestone, 1940.

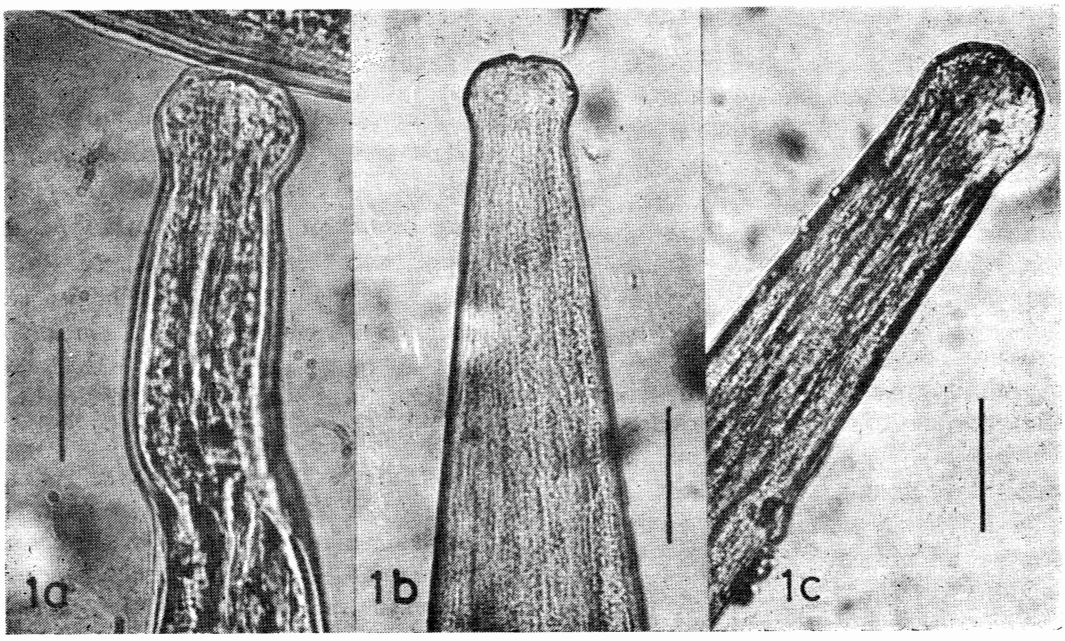

Fig. 1a—Anterior end of worm from patient's eye.
1b—Anterior end of adult *Brugia malayi*.
1c—Anterior end of adult *Wuchereria bancrofti*. Scale: 30 μm

Indian Med. Gaz., 75 : 159; Buckley and Edeson, 1956. *J. Helminth., 30* : 1).

The patient was amicrofilaraemic by direct smear and membrane concentration technique. Serological studies with the indirect fluorescent antibody test (IFAT) using papainized and sonicated microfilarial antigens of *B. malayi* and *B. pahangi* gave titres below 1 : 10. Using frozen sections of adult worms of these two species as antigens, the IFAT gave titres below 1 : 2. These negative results are expected as the female worm is non-gravid and located on the conjunctiva. It is tempting to postulate that the site of the worm could possibly be due to the site of bite by the infective mosquito as adult *B. malayi* worms have been recovered from the periorbital tissues of cats experimentally infected around the eye with infective larvae (Mak and Sivanandam, 1976. *Southeast Asian J. Trop. Med. Pub. Hlth., 7* : 21).

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A MODIFIED MEMBRANE FILTRATION TECHNIQUE FOR RECOVERY OF *WUCHERERIA BANCROFTI* MICROFILARIAE

Concentration methods are considered more sensitive for detection of low grade microfilariae (mf) than the conventional thick blood smear method, and are of special importance in the assessment of filariasis control programs (Knott, 1939. *Trans. Roy. Soc. Trop. Med. Hyg., 33*: 191; Bell, 1967. *Ann. Trop. Med. Parasit., 61*: 220, Chulererk *et al.*, 1970. *J. Parasit., 56*: 623; Dennis *et al.*, 1973. *Trans. Roy. Soc. Trop. Med.*

Hyg., 65: 521; Desowitz *et al.*, 1973. *Southeast Asian J. Trop. Med. Pub. Hlth., 4:* 179; Wang *et al.*, 1977. *Southeast Asian J. Trop. Med. Pub. Hlth., 4:* 324; Partono *et al.*, 1973. *Trop. Geog. Med., 25*: 286; Oemijati *et al.*, 1975. *Southeast Asian J. Trop. Med. Pub. Hlth., 6:* 186; McMahon *et al.*, 1979. *Ann. Trop. Med. Parasit., 73*: 457; Fan *et al.*, 1981. *Nat. Sci. Council Monthly, 9:* 1127).

Knott's method concentrations mf by concentration of a large blood sample (Knott, 1939) ; while, Bell (1967) recommended filtering hemolysed blood through a millipore filter. Both techniques are limited for use in the field, since electricity is required to carry out the procedures. To overcome this limitation, Chulererk and Desowitz (1970) devised a simplified membane filtration technique, which requires only a disposable syringe and a Swinnex membrane filter holder.

The membrane filter used in both Bell (1967) and Chulererk and Desowitz (1970) techniques must be dried, fixed, stained and washed outside of a Swinnex membrane filter holder. Therefore, some mf may be lost and additional time spent carrying out the process (Denham *et al.*, 1971. *Trans. Roy. Soc. Trop. Med. Hyg., 65*: 521). To rectify these problems, a modified millipore membrane filtration technique (MMMFT) has been developed and used for Bancroftian filariasis control program on Kinmen (Quemoy) Islands in the past 10 years (Fan *et al.*, 1981). The results of experimental trials are presented here.

One hundred and fifty (150) ml of venous blood were taken from a male (*Wuchereria bancrofti* microfilarial carrier volunteer (45 year old Kinmen Chinese) at 2300 h and mixed thoroughly with 0.63 gm of sodium citrate powder in a sterilized bottle (500 ml), and then transported by air to the Parasitology Laboratory, National Yangming Medical College in Taipei City.

The blood sample was poured into a beaker (500 ml) and shaken continuously during the process. One ml blood sample was removed from the beaker with a 10 ml disposable syringe, and the syringe equipped with a Swinnex 25 mm circular filter holder containing membrane filter with a porosity of 5 μ (Millipore Co. Bedford, Mass.). The membrane was supported beneath with a plastic circular pad.

Nine ml of 10% Teepol-saline solution were added and the syringe shaken gently and the solution allowed to clear. The hemolysed blood sample was subsequently expressed through the membrane filter by continuous pressure on the syringe piston. This was allowed by 10 ml of physiological saline for a rinse and 10 ml of warm water (50-60°C) to fix mf.

Five ml of 0.1% methylene blue solution were taken into syringe and gently forced through the membrane filter to stain mf for 10 minutes. The outlet of the Swinnex filter holder was blocked to prevent leakage of the stain with a disposable needle in its plastic protective cover (Fig. 1). After staining, 10 ml of physiological saline was passed through the filter for rinsing.

The membarne filter was removed from the Swinnex filter holder and placed onto a 3″ × 1″ microscope slide and dried at the room temperature. Immersion oil, 3-5 drops, were placed on the membrane filter and circular cover glass (25 mm in diameter) placed onto the oil. The mf were examined and counted under low power magnification (100X).

For comparison the MMMFT was evaluated along with the millipore membrane filtration technique (MMFT) of Chulererk *et al*, (1970). A total of 142 ml blood samples were available from the original 150 ml of blood

Fig. 1—A 10 ml disposable syringe equipped with a Swinnex circular holder containing a membrane filter (25 mm) with porosity of 5 micron, the outlet of Swinnex holder was plugged with a disposable needle in its plastic protective cover.

and alternate samples were evaluated by one of the methods. For the MMMFT odd numbered samples (1, 3, 5, 7 etc) were used and for the MMFT, the even numbered samples (2, 4, 6, 8 etc).

The number of mf detected by the MMMFT totaled 54,902 and individual counts ranged from 422 to 976. The counts for the MMFT totaled 46,663 and ranged from 235 to 962. The average number recovered was 773 for the former and 657 for the latter techniques. These data showed significant difference $p<0.05$ or 0.001 by student "t" test. The recoveries by the MMMFT was significantly higher than by the MMFT. The reason for the differences are probably due to the loss of mf during staining and rinsing processes outside of the Swinnex filter holder by the MMFT (Denham *et al.*, 1971).

In addition to higher recovery rate by the MMMFT, the time spent on the procedures was much less than that required for the MMFT, which requires time for drying. The drying time in both techniques, however, could be reduced by placing the microscope slide with the membrane filter over a low alcohol flame (Partono *et al.*, 1973).

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REDUCE EMBRYONATION OF *SCHISTOSOMA JAPONICUM* EGGS AS A CONTRIBUTORY MECHANISM IN MODULATION OF GRANULOMA IN CHRONICALLY SENSITIZED MICE

The major disease manifestation of schistosomiasis result from granulomatous inflammation and subsequent fibrosis around entrapped eggs in host tissues (Warren, 1973; *Helm. Ab., 42* : 591). During the course of acute experimental *Schistosoma mansoni* or *S. japonicum* infection, large granulomas are formed around eggs in the tissues (Domingo and Warren, 1968, *Amer. J. Path., 52* : 369 ; Warren and Berry, 1972, *J. Infect. Dis., 162* : 482). This in time is followed by modulation in chronic infection with formation of smaller granulomas (Warren, 1966, *Amer. J. Path. 49* : 477; Boros *et al.*, 1975, *J. Immunol., 114* : 1437). Similar modulation of lung granuloma formation around *S. japonicum* egg has been observed in a proportion of mice chronically sensitized by intraperitoneal injections of eggs for 16 weeks (Mitchell *et al.*, 1982, *Aust. J. Exp. Biol. Med. Sci., 60* : 410) and in unpublished experiments in mice sensitized with 28 to 32 weekly injection of eggs. Two mechanisms probably working in combination are likely to be involved in modulation of granuloma formation. The first is accelerated egg destruction (Colley, 1976, *J. Exp. Med., 143* : 696; Olds and Mahmoud, 1981, *Cell Immunol., 60* : 251) while the second mechanism is suppression of immunopathologic T-cells by suppressor T-cells (Phillips *et al.*, 1980, *Amer. J. Trop. Med. Hyg., 29* : 820; Chensue *et al.*, 1981, *J. Immunol., 127* : 363). Recently Olds *et al.*, (1982, *J. Immunol., 128* : 1391) modulated *S. japonicum* granuloma formation by serum transfer.

The purpose of this investigation was to determine if, in addition to the above mentioned mechanisms of modulation, reduced embryonation or inhibition of maturation of *S. japonicum* eggs in chronically infected or sensitized mice is a cause of modulation (Garcia *et al.*, 1981, *Southeast Asian J. Trop. Med. Pub. Hlth., 12* : 384). This hypothesis derived from our observation that *S. japonicum* eggs obtained by digestion of livers of infected rabbits at earlier or later than the 55th to 60th day of infection were less suitable for performance of the circumoval precipitin test (COPT). The unsuitability of eggs harvested at early time points of infection can be explained by immaturity of eggs while for later time points, this unsuitability may be due to inhibition or retardation of embryonation and accelerated destruction of eggs. Accelerated egg destruction together with inhibition or retardation of embryonation would prevent release of sufficient antigens from the egg needed for the formation of granulomas of large size.

To test our hypothesis, a comparison was made of the development of immature eggs injected intravenously into chronically egg-sensitized and unsensitized control mice. BALB/c mice, repeatedly sensitized by 28 to 32 weekly intraperitoneal injections of *S. japonicum* eggs, were injected thru the tail vein with 50 to 100 immature eggs from female worms freshly recovered by perfusion of the portal vein. Representatives of the sensitized group of mice were shown to have modulated granuloma response by radioisotopic assay (Mitchell *et al.*, 1982, *Aust. J. Exp. Biol. Med. Sci., 60* : 401). An age-matched control group of unsensitized mice were similarly injected.

Table 1 shows the result of enumeration and determination of the state of development of the eggs in the lungs of the chronically egg-sensitized mice and age-matched unsensitized mice. The results clearly indicate inhibition or retardation of maturation or

Table 1

Evidence for maturation arrest of eggs of *Schistosoma japonicum* in BALB/c mice sensitized by weekly intraperitoneal injections of eggs for 28-33 weeks.

Mice	No. of mice	No. of mice with < 25% mature eggs	Percentage of mature eggs in lungs*	
Egg-sensitized	11	8	25.8 ± 8.6	$p < 0.025^{+}$
Age-matched controls	11	3	59.8 ± 11.3	

*Mice were injected intravenously with 50 to 100 uterine eggs from *S. japonicum* adult worms and lungs examined for mature eggs (as distinct from immature and egg shells) at days 12 to 16. Mean numbers of eggs examined in each mouse of the egg-sensitized and control groups were 46.4±18.9 and 58.7±20.0 respectively. Examination was made by compressing the lungs between glass slides and microscopy.

+P values determined by the Mann-Whitney U statistics (Sokal and Rohlf, 1973).

embryonation. This result supports our hypothesis that a possible, and to date neglected, modulating mechanism of granuloma formation in the *S. japonicum*/mouse model is retardation or inhibition of embryonation. As stated earlier, modulation of granuloma against *S. japonicum* eggs has been achieved by transfer of serum of chronically infected mice to sensitized mice (Olds *et al.*, 1982, *J. Immunol., 129* : 1391). It is very possible that one of the effect of the antibodies presumed to be responsible for the serum effect in the experiment of Olds and co-authors is retardation of embryonation in addition to accelerated egg destruction. If this is so, probably an antibody-mediated immunologic blockade acts to prevent maturation of *S. japonicum* eggs in the tissues. This blockade may prevent the development and high rate release of the major granuloma producing antigens occurring with or as a result of egg maturation. As the multiple mechanims of granuloma formation and modulation become better understood, it should be possible to induce a "modulated state" before infection by way of vaccination and thus reduce the pathology of chronic infection.

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ASCORBIC ACID AND IRON EXCRETION IN A THALASSEMIC CHILD

Ascorbic acid (AA) as a pure form or as part of the multivitamins is always prescribed to thalassemic children. AA has been known to enhance the iron absorption. Our objective was to study the effect of this drug on the urinary excretion or the iron store (serum ferritin) in thalassemic children.

The study was performed by giving AA tablet orally 20 mg/kg daily to a 5-year-old boy with β thalassemia- hemoglobin E disease for one month, stopped for one month, and giving for one month alternatively for 3 paired-periods. Daily urinary iron excretion (24 hour collection) was determined by atomic absorption spectrophotometry (Graziano *et al.* 1974). The days of incomplete urinary collection were discarded. Urinary iron of 11-27 days for each period were compared between the period of taking and not taking AA. Serum ferritin was collected periodically to determine the amount of stored iron. The ferritin level was measured by a double antibody radioimmunoassay using kits (Clinical Assay Lab. Inc., Cambridge, MA., U.S.A.) according to the method of Yalow and Berson (1971).

The results are shown in Tables 1 and 2. There are no significant differences in the urinary iron excretion and the serum ferritin while the patient was taking or without taking AA. There were also no clinical changes during the period of the study.

From this study it was observed that AA did not have any effect on the urinary iron excretion or iron storage in a thalassemic child.

Table 1

The mean of each period of daily urine iron excretion.

Urine collection	Urine iron (mg per day)	
	Without AA mean ± SD	With AA mean ± SD
First	0.473 ± 0.424	0.369 ± 0.130
Second	0.615 ± 0.439	0.488 ± 0.306
Third	0.479 ± 0.198	- -
$\bar{X}$	0.522 ± 0.081	0.429 ± 0.084

No statistical difference ($p > 0.15$)

Table 2

Serum ferritin during the periods receiving AA compared to without AA.

Urine collection	Serum ferritin (mg/ml)	
	Without AA	With AA
First	337	336
Second	358	290
Second	385	389
Third	467	-
$\bar{X}$	387	338

No statistical difference ($p > 0.15$)

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VITAMIN K LEVEL IN MATERNAL BREAST MILK OF INFANTS WITH ACQUIRED PROTHROMBIN COMPLEX DEFICIENCY SYNDROME

Acquired prothrombin complex deficiency (APCD) syndrome or Idiopathic vitamin K deficiency in infancy is commonly found in 1-2 month old breast-fed infants. Bleeding symptoms, pallor and mild hepatomegaly are the major manifestations with a strikingly high incidence (65-100%) of intracranial hemorrhage. All of them exhibit severe prothrombin complex deficiency with normal platelet count (Bhanchet *et al.*, 1977. *Clin. Pediat., 16* : 992; Iizuka and Nago, 1979. *Blood* and *Vessel., 10* : 649).

The pathogenesis and definite etiology of this disease are still unknown, eventhough it has been described since 1966 from various parts of the world. Severe prothrombin complex deficiency is responsible for the bleeding diathesis and all patients respond well to small amount of vitamin K (Bhanchet-Isarangkura, 1979. *Southeast Asian J. Trop. Med. Pub. Hlth., 10* : 350).

Because 98% of infants who suffered from APCD syndrome were breast-fed, therefore, the objective of this study was to measure the vitamin K content in the breast milk of mothers of 9 infants with APCD syndrome in comparison with 13 samples of those with normal infants of the same age group. All mothers of both groups were healthy, had no special eating habits, and from the same socio-economic class.

The milk samples were stored at -30°C and sent in dry ice by airmail for vitamin K level measurement in Japan. Vitamin K determination was performed by high performance liquid chromatography combined with electrochemical detector (HPLC-ECD).

Table 1

Vitamin K content in maternal breast milk of APCD infants and normal infants.

No.	Level of vit K_1 mcg/l	
	Normal (13)*	APCD Syndrome (9)*
1.	14.9	7.8
2.	4.2	6.2
3.	7.3	2.3
4.	11.7	2.8
5.	9.8	1.5
6.	11.1	3.0
7.	6.8	7.6
8.	7.8	2.2
9.	6.9	4.1
10.	4.0	-
11.	4.4	-
12.	7.4	-
13.	16.0	-
Range	4.0-16.0	1.5-7.8
Mean	8.87**	4.17**
SD	3.67	2.42

* Number of cases.
** P value < 0.005.

The results of vitamin K content is shown in Table 1. Vitamin K level of APCD group was 4.17 ± 2.42 mcg/litre which is significantly lower than those of the normal group (8.87 ± 3.67 mcg/litre) $p = < 0.005$.

The result of this study suggested that low vitamin K content in the maternal breast milk of these infants may play a role in the etiology of this disease.

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ACETYLCHOLINESTERASE ACTIVITY IN RED CELLS INFECTED WITH *PLASMODIUM FALCIPARUM* CULTURE *IN VITRO*

It has been shown in a previous report that red cells in patients infected with *Plasmodium falciparum* or patients at the convalescent stage contained normal amount of acetylcholinesterase (ACHE) activity (Areekul *et al.*, 1982. *Southeast Asian J. Trop. Med. Pub. Hlth. 13* : 196). After partial separation of parasitized erythrocytes from non-parasitized erythrocytes from infected blood cultured *in vitro* for 24 hours, mature asexual erythrocytes forms in the upper portion contained a relatively higher ACHE activity while ring form red cells had a similar ACHE activity as the normal red cells. Furthermore, a reverse relationship between red cell ACHE activity and the parasitaemia was also demonstrated in the upper portion. In order to investigate on the higher parasitaemia and more mature asexual erythrocytes forms, the present study was undertaken to determine ACHE activity in red cells infected with *P. falciparum* malaria after culture for a longer period.

The study was carried out on 6 patients suffering from *P. falciparum* malaria as well as in 6 control subjects. Venous blood samples were collected in a test tube using EDTA as an anticoagulant. One ml of packed red cells was mixed with 11 ml. of RPMI 1640 and kept in culture continously for 8 days by using the candle jar method as described by Jensen and Trager (1977. *J. Parasitol., 63* : 883). Blood samples were taken each day for partial separation of parasitized erythrocytes from non-parasitized erythrocytes by using 5% Ficoll solution (Mrema *et al.*, 1979. *Bull. WHO., 57* : 133). Red cell ACHE activity in each sample was determined by the micro method (Ellman *et al.*, 1961. *Biochem. Pharmacol., 7* : 88).

Results showed that the ACHE activity of the red cell from normal subjects were stable throughout the period of study for 8 days. In the infected blood, there was an elevation of parasitaemia in all portions of blood with marked changes in the upper portion. i.e., 14/1000 red cells on day 0, 104/1000 red cells on day 6 and 107/1000 red cell on day 8. As the parasitaemia increased, the ACHE activity in this portion decreased continuously and showed significant difference from those of normal red cells on day 3 to day 5 as shown in Fig. 1. On the other hand, ACHE activity in red cells of the lower portion and blood samples before separation showed no significant deviation from the normal red cells.

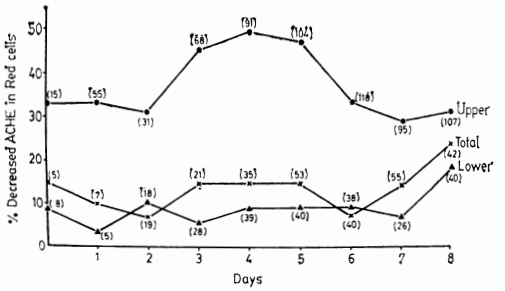

Fig. 1—Showing the mean values of % decreased ACHE activity in red cells in the upper (U), lower portions (L) and blood samples before separation (T) from 6 patients and 6 control subjects. The parasitaemia per 1000 red blood cells, is shown in parenthesis.

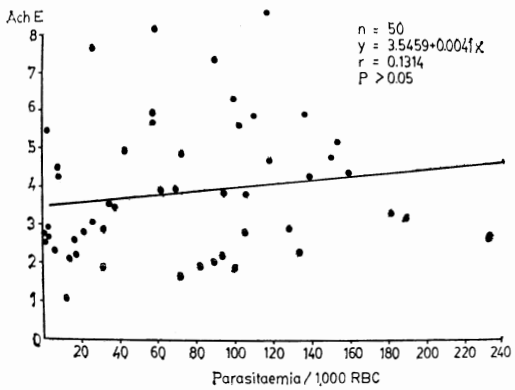

Fig. 2—Showing the relationship between the ACHE activity (I.U) and the parasitaemia in the upper portion of blood samples.

Fig. 2 illustrates the relationship between parasitaemia and red cells ACHE in the upper portion.

The present study shows that ACHE activity of infected red cells on day 0 and day1 were not significantly different from those of normal red cells. These findings were in accordance with results reported earlier (Areekul *et al.*, 1982. *Southeast Asian J. Trop. Med. Pub. Hlth., 13* : 196). Findings of a significantly decreased ACHE activity only in the upper portion on day 3 to day 5 indicated that the mature asexual erythrocytes forms contained less ACHE than the ring form red cells or normal red cells. The mechanism of reduced ACHE activity in red cells infected with this stage of *P. falciparum* has not been reported and therefore a speculation could only be made. It was highly probable that the mature asexual form of malaria inactivated and/or utilize ACHE of the infected red cell membrane. The presence of ACHE in malarial parasite has not been clearly demonstrated, but this did not rule out the above probability. The presence of malaria may also interfere with the synthesis of this enzyme protein in the erythrocyte membrane, but as the last probability. Studies on these mechanism are in progress in this laboratory.

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TRAPPED RED CELL VOLUME IN RHESUS MONKEYS INFECTED WITH *PLASMODIUM KNOWLESI*

It has been shown earlier that there was an increased erythrophagocytic activity in rhesus monkeys infected with *Plasmodium knowlesi* and this activity returned to normal value after being treated with anti-malarial drugs (Areekul *et al.*, 1973. *Southeast Asian J. Trop. Med. Pub. Hlth., 4* : 598). However, an attempt to demonstrate the trapped red cells in the brain of rhesus monkeys infected with *P. knowlesi* by injecting ^{51}Cr-labelled infected red cells and ^{59}Fe-labelled normal red cells was not successful (Areekul *et al.*, 1972.

Southeast Asian J. Trop. Med. Pub. Hlth., 3 : 617). The present investigation was undertaken in order to demonstrate this phenomena in rhesus monkeys infected with *P. knowlesi* by using other approaches.

A rhesus monkey (*Macaca mulatta*) was injected intravenously with a ^{59}Fe-ferric citrate solution containing 500 microcuries of ^{59}Fe. Three weeks after injection, blood was withdrawn and centrifuged and the ^{59}Fe-red cells were used as the normal red cells.

Another rhesus monkey was infected with *P. knowlesi*. Ten days after infection, blood with malarial infection rate of 5-20% was taken and red cells were labelled with ^{51}Cr by the ordinary method. The mixed solution of ^{51}Cr-labelled infected red cells and ^{59}Fe-labelled normal red cells was injected intravenously into the experimental monkeys. Blood samples were taken at 10, 20, 30, 40, 60, 80, 100 and 120 minutes after injection for determination of hematocrit and radioactivity of ^{59}Fe and ^{51}Cr. The samples were assayed in a well type scintillation counter connected to a pulse height analyzer, so that the radioactivity of ^{51}Cr and ^{59}Fe could be counted separately.

Red cell volume (RCV) was calculated from a formula :-

$$RCV = \frac{D \times Ht \times 0.96}{A} \quad \ldots\ldots\ldots\ldots (1)$$

where D=dose given, Ht=haematocrit (%), A=radioactivity of the sample. Per cent of trapped red cell volume was calculated from a formula :-

$$\% \text{ trapped RCV} = \frac{RCV_{Cr} - RCV_{Fe}}{RCV_{Fe}} \times 100 \quad \ldots\ldots\ldots\ldots (2)$$

where RCV_{Cr} and RCV_{Fe} were red cell volumes calculated from ^{51}Cr-labelled red cells and ^{59}Fe-labelled red cells respectively.

Table 1

Showing the mean % trapped red cell volume after injection of ^{51}Cr-infected RBC and ^{59}Fe-normal RBC in normal and infected monkeys.

Monkeys	Ht (%)	Red cell volume determined by ^{59}Fe	Red cell volume determined by ^{51}Cr	Trapped red cell volume (%)	Parasitaemia (per 1000 RBC)
Normal					
M-3	39	34.86 (33.24-36.65)	34.00 (33.78-36.34)	-2.44	0
M-7	41	35.24 (34.06-37.01)	34.84 (33.82-36.10)	-1.10	0
M-32	37	48.48 (44.02-50.95)	54.91 (53.53-58.11)	+13.14	0
Infected					
M-32	32	37.32 (33.45-40.90)	41.95 (38.11-44.56)	+15.80	53
M-29	16	15.23 (13.72-16.67)	20.07 (19.18-21.61)	+32.09	22

Range shown in parenthesis.

The studies were performed on 3 normal monkeys and 2 monkeys infected with *P. knowlesi* with the parasitaemia of 22/1000 and 53/1000 red cells, respectively. Results of % trapped RCV in these monkeys are shown in Table 1. Less than 5% of trapped RCV were detected in 2 normal monkeys (M-3, M-7) while a value of 13% was found in the third one (M-32) which resulted in a mean value of 3.2% in this normal group. Higher values of 16% and 32% trapped RCV (average 23.9%) were found in 2 infected monkeys. The changes of % trapped RCV during the whole course of studies are illustrated in Fig 1.

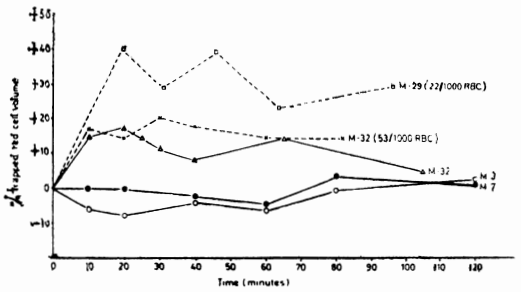

Fig. 1—Showing the changes of % trapped RCV in 3 normal monkeys (M-3, M-7, M-32) and 2 *P. knowlesi* infected monkeys (M-29, M-32) with parasitaemia of 22/1000 and 53/1000 red cells respectively.

Results in the present study showed that RCV calculated from ^{51}Cr and ^{52}Fe in 2 normal monkeys were quite close together. This indicated that both infected and normal red cells labelled with these two radioisotopes were not damaged by the procedure of labelling techniques. Findings of a mean value of 3% trapped RCV in normal monkeys compared to 24% in infected monkeys indicated that the infected erythrocytes labelled with ^{51}Cr were removed more rapidly from the circulation than uninfected erythrocytes labelled with ^{59}Fe. This accelerated clearance appeared to be much higher in infected monkeys than in normal monkeys. These findings were in accordance with results reported earlier that the clearance of ^{51}Cr-labelled *P. berghei* infected erythrocytes was faster than uninfected erythrocytes in rats (Quinn and Wyler, 1979. *J. Clin. Invest., 63* : 1187). The enhanced clearance of the parasitized erythrocytes was possibly due to the removal of these erythrocytes by the spleen. Studies with transfused ^{51}Cr-labelled red cells from monkeys infected with *P. knowlesi* showed that the spleen of recipient monkeys removed parasites from the transfused cells without destroying them (Conrad and Dennis, 1968. *Amer. J. Trop. Med. Hyg., 17* : 170). This was supported by an electron microscopic evidence of pitting of the portion of erythrocytes containing malarial parasites in the spleen of rhesus monkeys infected with *P. knowlesi* (Schnitzer *et al.*, 1973. *Blood, 41*: 207).

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